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REPORT OF INVESTIGATIONS

INTO THE

PATHOGENY OF DIPHTHERIA,

CONDUCTED BY

EDWARD CURTIS, M. D. AND THOMAS E. SATTERTHWAITE, M. D.

NEW YORK, February 11, 1877.

To the Board of Health:

GENTLEMEN—I have the honor to submit herewith a report of investigations made in accordance with a resolution of the Board of Health, passed November 24, 1874, as follows:

"*Resolved*, That Dr. Edward Curtis be requested, with the concurrence and under the supervision of the Sanitary Committee, to investigate the causes and nature of diphtheria by means of micro-pathological examinations and otherwise."

At my request Dr. Thomas E. Satterthwaite was associated with me in the investigation, and the experiments made were conducted by us jointly.

Very respectfully, your obedient servant,

EDWARD CURTIS, M. D.,
Honorary Microscopist to the Board.

PART I.—GENERAL REPORT.

By the broad instructions to investigate the "causes and nature of diphtheria," we conceived that we were desired to work at some point in the pathogenesis of the disease, whose solution was important and at the same time seemingly within the means of laboratory experimentation. Accordingly we took for our subject the question which has already attracted so much attention from its supposed solution in Germany, viz: *what is the nature of the infectious principle of diphtheria, and what are the circumstances that determine the infection.*

Considering it already sufficiently proven that the false membrane of diphtheria is able to communicate the disease, we made our search for the infectious principle in this material. The first point was to know the morphological elements of the membrane. Concerning most of these there has never been any doubt; fibrin, epithelial cells, leucocytes and granular matter being easily recognizable. These elements we found in varying proportion in most of the sixteen[1] membranes examined by us, and in three[2] there was also the mycelium, and in one[3] of the same also the spores of a fungus, apparently an aspergillus or penicilium. But the important point was to determine the presence or absence in the membranes of *bacteria*, since these organisms have been alleged to be not only always present, but also to contain or be the noxious element. Accordingly we searched our membranes with special care for bacteria, with the following result: Small rod-bacteria, precisely similar in form and size to those found upon the mucous membrane of the healthy mouth, we found almost always present, though in greatly variable quantities, scattered throughout the tissue of the false membrane. Besides these rod-forms there also occurred in many, though not in all, of our membranes, distinct collections of closely packed bacteria, apparently either firmly glued together or else encased in a delicate invisible envelope. For these collections, or "balls" or "nests," could be isolated and rolled about and subjected to heavy pressure by pressing upon the cover of the microscopical slide, without losing their form or coherence. Similar balls or masses have been repeatedly seen by us in non-diphtheritic matter infested with bacteria. On treating membrane containing these balls with an alkali, the balls disappear as such, and the field becomes crowded with short rod-bacteria. From this fact it is likely that these balls are not, as their appearance first suggests, wholly collections of *spherical* bacteria, but that much of their contents at least is the ordinary rod-form of bacteria, which here looks spherical simply because the individuals are closely packed side by side with their ends presented to view. The same illusion of minute spheres is commonly met with wherever, as upon the papillæ of the healthy tongue, rod-bacteria are closely massed together. In addition, free spherical granules presenting the optical characteristics of bacateria were often met with, but rarely except in company with the free rods and the balls of massed bacteria already described. Upon these granules we tried the various chemical and coloring tests for bacteria proposed by Hiller, Letzerich and Eberth, but we found the tests so unreliable that we dared not base conclusions upon their showings. Inasmuch furthermore as the granules in question were almost always associated with unmistakable bacteria, and were in no wise different in appearance from the similar granules found commonly in all bacteria-infested matter, whether diphtheritic or not, we did not attach much

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[1] Several specimens were examined which do not appear in Part II., because no use was made of them for experimentation.

[2] Cases VI., XI., and XIII. See Part II., pp. 677, 689, 690.

[3] Case XI.

importance to the determining whether or not the granules were themselves bacteria. Whenever, therefore, it may be remarked in passing, statements are made in this report about *bacteria*, either rods or balls of massed bacteria—both definitely recognizable by the microscope—are always meant.

So far then as regards the presence of bacteria in our diphtheritic membranes, we found abundant evidence of the existence of the form described by other observers, but these forms were in no wise different in optical or chemical behavior from the bacteria found in putrescent but non-diphtheritic animal matters.

The morphological elements being thus determined, and being found to present nothing *sui generis* in form, we proceeded to our proposed experimentation, which was to inoculate living animals with diphtheritic membrane under varying conditions. The idea was that if, as alleged,[1] inoculation will produce diphtheria in the inoculated animal, then, by varying the conditions, the nature of the poisonous principle and the determining circumstances of infection might be discovered. The animal chosen for experimentation was the rabbit, partly because we had not the facilities for dealing with larger or more troublesome animals, and partly because some German investigations, with which it was desirable that ours might be compared, were also conducted upon rabbits.

The first point was to determine, as a basis for comparison, the effect upon the rabbit of simple inoculation of fresh diphtheritic membrane. Following Eberth,[2] we first tried inoculating the cornea. This was done by pricking and scratching the cornea with a needle, then rubbing into the abrasions a bit of diphtheritic membrane, and finally holding the lids shut for a few minutes with the piece of membrane inside. According to Eberth[3] this proceeding begets an extensive diphtheritic keratitis with constitutional symptoms and even termination in death. In our hands, however, nothing resulted but small local spots of inflammation at the site of each puncture, which appeared the day after infection, and speedily subsiding, left the eye well by the fourth or sixth day. No severe constitutional symptoms appeared, and none of the animals died except one whose thigh was also inoculated. These inoculations were tried with four[4] different diphtheritic specimens. In three[5] of these it is an open question if the material inoculated was active, but in the third[6] case there is no such doubt, for the same membrane, which was thus almost wholly innocent upon six corneæ, produced extensive lesions and proved fatal in forty-five hours when inoculated into the muscular tissue of the thigh.[7] On account of these failures we abandoned trying the cornea, and in all subsequent inoculations resorted to the muscular or subcutaneous connective tissue. Where the material to be inoculated was solid, as with bits of diphtheritic membrane, it was inserted into the muscular tissue either of the fore or hind leg, by the following method: A snip is made through the skin and subcutaneous connective tissue an inch or more distant from the intended site of inoculation in the muscle; then the skin is drawn along till the hole exposes the muscle, when a cut is made into the latter tissue, and the inoculating material pushed into the wound. The skin being then let go, resumes its natural position, and the inoculated substance is retained in place without the need of a suture. Where the inoculating material was fluid it was simply injected subcutaneously by an ordinary hypodermic syringe. Thirty-eight rabbits

1 Oertel, Eberth and others.

2 Zur Kenntniss der Bacteritischen Mykosen. C. J. Eberth, Leipsig, 1872.

3 Op. cit.

4 Cases I., II., III., IV. See Part II., pp. 671, 672, 673, 675.

5 Cases I., II., and IV.

6 Case III.

7 Experiment 8. See Part II., p. 674.

were thus inoculated with pure diphtheritic membrane from twelve different specimens.[1] Of these animals twenty-seven were inoculated with bits of solid membrane, the size of the pieces varying with the amount of material on hand, but varying from one-sixteenth to one-fourth inch square. The other eleven animals were injected with aqueous infusion of the membrane, made by cutting the latter up fine, thoroughly triturating it with cold water, and then injecting the turbid infusion after the larger pieces of membrane had settled. Of the inoculations with solid matter all but three[2] were double, that is, were made upon both the opposite legs of the animal, hind or fore, as the case might be. Of the infusion injections all but four[3] were similarily double. Of the thirty-eight animals inoculated, four[4] showed no effects whatever, local or constitutional. Of these, three[5] had been inoculated with the membrane of Case IV.,[6] which was a soft, glutinous material, made up of leucocytes and granular matter imbedded in mucus. It had formed in the nose of a three-year-old child on the second or third day of an acute nasal catarrh, and had become detached a day or two later, the child recovering without any alarming symptom. Both the appearance of the membrane and the history of the case thus made it doubtful if the disease in this instance were really diphtheria. All the other animals, inoculated, thirty-four in number, developed local lesions at one or both sites -generally both—of varying degrees of intensity. The great majority also showed constitutional symptoms, and twenty-two died. One death,[7] however, is doubtful, as the rabbit was found half out of his cage, wedged between the bars, and may have injured himself. But this case is fairly offset by a doubtful recovery,[8] where the local lesion was bodily removed on the fifth day, and the animal slowly recovered, whereas, four[9] other rabbits inoculated with the same membrane and left untouched, all died. Of the twenty-one genuine deaths, five[10] took place between the first and second day after inoculation, thirty hours after being the earliest period—six[11] between the second and third day, one each on the third,[12] fourth,[13] sixth,[14] fourteenth,[15] nineteenth,[16] and twenty-first,[17] two on twenty-third,[18] and one each on the thirty-first[19] and thirty-eighth[20] days.

Our results thus confirmed those of other investigators, namely—that the matter of diphtheritic membranes inoculated into the muscular tissue of rabbits proves poisonous, and in the majority of cases fatal. But it still remained to be determined whether, as alleged, the disease produced, be actually diphtheria. To decide the question the first step obviously was, to compare the rabbit-disease produced by these inoculations with diphtheria as it occurs in the human subject. A study of our cases showed that the inoculation disease, though differing greatly in severity, course and results in different instances, yet was always the same process, as follows: At varying periods after the inoculation a lump appears under the skin at the inoculated spot. On cutting down upon it, a yellowish white mass is exposed protruding from the wound in the muscle into the connective tissue in the line of communication between the original skin and muscle-openings. When first formed, this mass is homogeneous, of pasty consistence, and when examined microscopically is seen to consist of the loose substance of the

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1 Cases III. to XIV. inclusive.
2 Experiments 12, 13 and 24.
3 Experiments 14, 31, 32, 33.
4 Experiments 12, 13, 14, 60.
5 Experiments 12, 13, 14.
6 See Part II., p. 675.
7 Experiment 62. See Part II., p. 691.
8 Experiment 23. See Part II., p. 679.
9 Experiments 21, 22. 24.
10 Experiments 8, 17, 18, 19, 39.
11 Experiments 20, 45, 52, 53, 54, 63.
12 Experiment 40.
13 Experiment 36.
14 Experiment 35.
15 Experiment 58.
16 Experiment 22.
17 Experiment 21.
18 Experiments 25, 68.
19 Experiment 26.
20 Experiment 24.

connective tissue densely infiltrated with leucocytes, and also with greater or less numbers of bacteria according as the original inoculated material was rich or poor in these elements. Around this patch or lump of infiltrated connective tissue there will be found more or less evidence of an irritant action. In the severest cases there will be intense hyperæmia of all the circumjacent parts, ecchymotic spots and extensive serous infiltration. In the animals that die early, these lesions are especially prominent, and in some cases death occurs before a lump has time to form at all.[1] But in cases not speedily fatal, the symptoms of great irritation are generally wanting, and the lump produced by formation of leucocytes develops slowly and apparently without any affection of the general health of the animal. In such case there will be but comparatively few bacteria to be found in the lump. The course of the disease in these cases follows two types. Either the lump, generally of small size, slowly disappears by absorption, leaving nothing but a cord of thickened connective tissue, or the material of the lump softens by fatty degeneration; the skin gives way, and a running sore is established with more or less burrowing of pus into the adjoining tissues. When the latter course develops itself, the animal frequently becomes emaciated; its fur unclean and mangy; it refuses food, and finally dies after a greater or less period, often extending over several weeks, with all the signs of general exhaustion. In cases where running sores and extensive burrowings of pus take place, the matter commonly becomes offensive and swarms with bacteria. In all types of the disease, there is a notable absence of lesions of any distant part. No exudations appear either upon any of the mucous membranes or upon any wounds which may be present, and there is no constant or even frequent lesion of any organ. In many cases we examined the blood, post-mortem, for bacteria, and while these bodies were found in about half the cases examined, their presence did not seem specially related to the presence of bacteria in the local lesion, nor did they occur more frequently than in the blood of non-inoculated animals, dead an equal length of time.

In the above described disease we failed to see anything specifically resembling diphtheria as it occurs in the human subject. The whole story seemed to be one of local irritant poisoning, which always tended towards the production of an abscess at the site of inoculation, with greater or less concomitant hyperæmia, ecchymoses, and serous infiltration of neighboring tissues, according to the degree of virulence of the inoculated poison. According, also, to the severity of the primary lesions, the animals would die or survive the immediate effects; and according to the secondary history of the mass infiltrated with leucocytes—according, namely, as the mass was re-absorbed or softened and ulcerated—the animal survived unaffected, or slowly wasted away and died of exhaustion. But it might, not without reason, be argued that a rabbit is a widely different animal from a man, and that a disease induced by subcutaneous inoculation might naturally differ in its manifestations from the same, occurring idiopathically; hence, that the described affection of rabbits might still be true diphtheria, although wanting in the specific characteristics of that disease as seen in the human subject. To determine this point we proceeded to try if effects similar to the foregoing would follow the inoculation of a material resembling diphtheritic membrane in its anatomical and chemical character, but yet not only *not* diphtheritic, but even incapable of producing any noxious effect under circumstances where diphtheritic membrane often proves highly infectious. Such a material presented itself in the *scrapings from the upper surface of a somewhat "furred" tongue from a healthy person.* These scrapings, while obviously not infectious when brought in contact with the pharyngeal mucous membrane, yet contain anatomical

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[1] See Experiment 8. Part II., p. 674.

elements of similar character, and in similar vital condition, to those of the diphtheritic membrane. They also swarm with countless bacteria.

Such scrapings, inodorous when fresh, acquire a peculiarly offensive fœtid smell even within a few hours. Inoculations with this pulpy material were then made in the usual way, with the following results: Three animals were inoculated with the matter freshly removed.[1] Of these, one[2] died between the fourth and fifth day with a lump in each thigh, in all respects similar to that produced by the diphtheritic inoculation. One[3] died on the fourteenth day, with similar lumps, which had softened and ulcerated; and the third[4] on the twenty-fifth day, with widely extended burrowings of pus and secondary peritonitis. Four other rabbits[5] were inoculated with tongue-scrapings twenty-four hours old and fœtid. Of these, the first[6] developed a large lump of the usual character in twenty-four hours. This was then removed entire for study, and the animal survived with no further lesion. The other three animals were let alone; all developed the usual lesions, and all died on respectively the fifth,[7] nineteenth,[8] and twenty-fifth[9] days. A third series[10] of two rabbits were inoculated with the mixed scrapings of five successive mornings, which was exceedingly offensive to the smell. One[11] died on the sixth day with the usual lumps, but as this was a young animal from a litter some of which died without having been experimented on at all, it is an open question whether or not the death in this case was due to the inoculation. In the other rabbit[12] lumps developed, which ulcerated and discharged foul matter. The animal suffered also in its general health, but ultimately recovered.

These series of experiments thus developed the important fact that certain non-diphtheritic and non-infectious matters will, when inoculated upon the rabbit, produce a disease closely similar to, if not identical with, the disease caused by the inoculation of diphtheritic membrane, and one, also, equally fatal in its effects. But inasmuch as tongue-scrapings are open to the suspicion of containing some peculiar poison derived from the animal body, we determined to experiment with a substance which should be free from such objection, while still resembling diphtheritic membrane and tongue-scrapings in the matter of being putrescent and swarming with bacteria. We accordingly chose *Cohn's fluid*, which had passed into a state of decomposition. This fluid is simply a solution in distilled water of ammonium tartrate, potassium phosphate, and magnesium sulphate, to which also a little calcium phosphate is added.[13] Upon exposure in a warm place, bacteria appear in this fluid in great numbers, and out of the salts present new compounds are formed of a strongly putrid smell. Inoculations were made with this putrescent material, as follows: Two animals[14] were inoculated in the usual way with the pulpy sediment forming at the bottom of the bottle, and obtained by straining the fluid. Both developed the usual purulent lumps, but with little attendant irritation or constitutional disturbance. In both, the lumps gradually disappeared by absorption and atrophy, and the animals survived. Four rabbits[15] were inoculated by hypodermic injection with the lower stratum of liquid in another sample of the decomposed fluid. Lumps, as usual, formed at the site of each injection; one animal died on the tenth day, the other three sur-

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[1] Experiments, 73, 74, 75. See Part II., pp. 694, 695.
[2] Experiment 73.
[3] Experiment 75.
[4] Experiment 74.
[5] Experiments 76–79. See Part II., pp. 695, 696.
[6] Experiment 76.
[7] Experiment 79.
[8] Experiment 77.
[9] Experiment 78.
[10] Experiments 83, 84. See Part II., p. 698.
[11] Experiment 84.
[12] Experiment 83.
[13] Ammonium tartrate, 1.0; potassium phosphate, magnesium sulphate, aa 0.5; calcium phosphate, 0.05; distilled water, 100 c. c.
[14] Experiments 91, 92. Part II., p. 700.
[15] Experiments 93–96. Part II., p. 701.

vived. Four[1] rabbits were injected hypodermically with the upper stratum of liquid in still another sample. Lumps again appeared at each inoculated spot, but they were small, and all the animals survived. The same procedure in four[2] other rabbits with a fourth sample of fluid produced like effects in three of the animals, but in the fourth no lumps discoverable by feeling the spot, could be found. Fourteen animals in all were thus inoculated with the putrid Cohn fluid. Thirteen of them developed lesions identical in appearance with the characteristic lesions from inoculations with diphtheritic membranes and tongue-scrapings, but the collateral effects were not so severe, and only one animal[3] died from the effects of the inoculation. In short, then, putrid Cohn's fluid, inoculated upon the rabbit, poisons after the same *manner* as diphtheritic membrane, though not to the same *measure*.

It would seem therefore that the disease produced in the rabbit by inoculations of diphtheritic matter, is not only not specifically diphtheritic in character, but not even peculiar to the diphtheritic infection; since a disease essentially similar, if it be not pathologically identical, is produceable, though in variable intensity, by inoculations of material at once non-diphtheritic and non-infectious to human mucous-membrane; and even, it may be, not of animal origin. This fact being apparently established, the important corollary follows, that pathological and pathogenetic conclusions drawn from the effects of diphtheritic inoculations of the rabbit, do not *of necessity* apply to the disease diphtheria as it appears in the human subject. The many current hypotheses in the premises therefore, which rest upon such animal experimentation, are built upon a quicksand.

This conclusion being accepted, it was plain that to attempt to elaborate the *pathology of the diphtheritic process*, by study of the rabbit's inoculation-disease, would be a waste of time, and all thoughts of the same were accordingly abandoned. But though the inoculation-disease is not necessarily, nor even probably, diphtheria, yet it is possible, and indeed probable, that the poisonous element in the diphtheritic membrane, which will produce diphtheritic infection in man, is the same *kind of thing* as that which produces the inoculation-disease in the rabbit. Hence, it seemed to us to be a useful research to try to discover the nature of the infecting principle, whether of diphtheritic membrane, tongue-scrapings, or Cohn's fluid, which produces the inoculation-disease.

It was, in the first place, conceivable, though not likely, that the disease in question might be due simply to mechanical irritation. To decide this point, we sought an inoculating material which should combine physical roughness with freedom from poisonous property. These qualities being obviously present in *sand*, we inoculated two rabbits[4] with dry sand, after the usual method, and two others[5] with a pulp made of sand mixed with Liebig's meat-extract. Not the slightest symptom, local or constitutional, followed in any of the cases. At various subsequent dates, the tissues in the track of the original wound in the various animals were exposed and examined. The grains of sand were found scattered in the connective tissue; this tissue was slightly thickened, but there was no trace of suppuration or other sign of irritation. It was seemingly evident therefore, as expected, that the cause of the inoculation-disease is other than mere mechanical irritation. The next point to be determined was, whether the poisonous principle is in solution in the fluid of the inoculating materials, or whether it resides in the solid elements of the same. For this inquiry, aqueous infusions of the infecting matters were filtered, and comparisons made of the effects of inoculation of the filtered and unfiltered fluids. Filtrations were made through various thicknesses

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[1] Experiments 97-100. Part II., pp. 701, 702.

[2] Experiments 106–109. Part II., pp. 703, 704.

[3] Experiment 94.

[4] Experiments 121, 122. Part II., p. 706.

[5] Experiments 123, 124. Part II., p. 706.

of ordinary filtering paper, and also through porous clay. The experiments with paper-filtered fluids were as follows: An infusion of diphtheritic membrane[1] was made and filtered through a double paper-filter.[2] The filtrate, which was clear and odorless, was injected hypodermically into three rabbits.[3] All survived, two without even any local lesion, but the third with a large lump of the usual character, containing living bacteria, upon one side. None of the animals seemed to suffer in general health. At the same time the unfiltered infusion was injected into three other rabbits,[4] and all of them died before the end of the third day with severe lesions. With another sample of membrane,[5] one rabbit[6] was inoculated with the filtered infusion, and one[7] with the original solid membrane. The former escaped with scarcely any effect, local or general, the latter died in forty-five hours with extensive and severe lesions. An infusion of a third membrane[8] was allowed to putrefy for ten days, and was then filtered through a single paper filter. The filtrate contained living bacteria, and retained the putrid odor; but it produced no effect at all upon injection into two rabbits.[9] The unfiltered fluid was also injected into two rabbits,[10] but produced only slight lesions. One of these animals died the day after the inoculation, but it had jumped from its cage and was found dead on the floor, and it is altogether probable that death was caused by the fall. A filtered infusion of still another membrane[11] was injected into a single animal,[12] but since in this case the original membrane, upon inoculation, proved to be itself innocuous, this experiment is to be disregarded.

As may be at once seen, the general result of the above inoculations was, that paper-filtration tended to diminish the poisonous virulence of the infusions. But the experiments were not thorough, for the filtration was insufficient to entirely remove certain of the solid constituents (bacteria) from the infusions. The difficulty was that, from the small quantities of diphtheritic membrane at our disposal, we could never obtain a large enough bulk of strong infusion to permit of repeated paper-filtration, such as alone would separate, with reasonable thoroughness, all the solid elements of the infusion. We were forced therefore to turn to other infectious infusions, where the requisite bulk of fluid could be had to test the matter thoroughly. We accordingly took putrid Cohn-fluid and filtered it as follows, fresh filters being used at each successive filtration: Twice through single filters of ordinary gray filtering-paper; twice through double filters of the same; once through a triple, and once through a quadruple filter; and finally, thrice through quadruple filters of fine, white filtering-paper. The final filtrate, instead of the milky appearance of the original fluid, was but faintly opalescent, and, what was interesting and suggestive, had entirely lost the strong putrid smell of the non-filtered fluid. Under the microscope (one-tenth inch immersion objective of J. Beck), the fluid appeared perfectly clear, except that about half a dozen small bacteria were to be found in each microscopic field. This filtrate was then injected into four rabbits,[13] four other rabbits at the same time receiving injections of the unfiltered fluid.[14] The latter animals all developed the usual lumps at each site of injection, but all those injected with the filtrate remained absolutely unaffected. No lumps whatever could be felt upon the thighs at any period; and, to make sure, the skin over one thigh was snipped on the fourteenth day, and through the "window" thus made, the whole subcutaneous territory was thoroughly inspected.

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[1] Case X., Part II., p. 687.
[2] Part II., p. 689.
[3] Experiments 55–57.
[4] Experiments 52, 53, 54. Part II., p. 688.
[5] Case III., Part II., p. 673.
[6] Experiment 11.
[7] Experiment 8.
[8] Case IX., Part II., p. 685.
[9] Experiments 48, 49.
[10] Experiments 46, 47.
[11] Case IV., Part II., p. 675.
[12] Experiment 16.
[13] Experiments 102–105., Part II., p. 703.
[14] Experiments 97–100., Part II., p. 701, 702.

Not the slightest lesion was to be found; it could not have been told that the region had been inoculated at all. Here, then, we seemed to have positive proof, so far as the experiments went, that thorough paper filtration would remove entirely the infectious element of the putrid Cohn-fluid. But here the following possible source of error was acutely pointed out by a friend.[1] During the very numerous filtrations above described, the fluid, falling slowly from the funnel drop by drop, had been submitted to a very thorough *aeration* as well as filtration; and, since it was conceivable that the infectious element might be a volatile chemical compound, it might be that in the experiment the innocuousness of the filtrate resulted from dissipation of a volatile principle by the aeration, instead of separation of a solid element by the filtration. To determine this point, a funnel was plugged with a rubber stopper pierced with a small hole, through which fluid could pass only slowly and drop by drop. Some putrid Cohn-fluid was then passed through this funnel as many times as the number of filtrations of the sample used in the last-described experiment. This aerated but *unfiltered* fluid was then injected into four rabbits,[2] four other rabbits[3] being at the same time injected with the original fluid fresh from the bottle. Lumps were produced at each site of injection in the rabbits treated with the aerated fluid, exactly as in those injected with the non-aerated. This test experiment thus removed the possible flaw in the evidence obtained by the filtration injections, and left no doubt that by the separation of the solid elements of infectious infusions by thorough filtrations, the infectious principle tends to be removed. Further corroboration was afforded by experiments with a putrid infusion of calf's liver. Injections of the unfiltered infusion[4] produced lumps as usual, but the fluid, when filtered,[5] even through a single filter, failed to produce any effect.

Simultaneously with the above described experiments with paper-filtrates, injections were practiced with some infusions filtered through porous clay. To obtain such filtrates, a porous clay battery-cylinder was made use of. A piece of wide rubber tubing, connected with an exhausting syringe, was slipped over the closed end of the cylinder, and by exhausting the air in the tubing, a little of any fluid in the clay cylinder would be drawn through the bottom of the same, of course suffering very perfect filtration in the passage. Infusions of three separate diphtheritic membranes,[6] a sample of putrid Cohn fluid and the infusion of calf's liver, already mentioned, were severally thus filtered. In all cases the filtrate was perfectly clear, entirely free from odor, and, examined microscopically with a 1-10-inch object-glass, apparently totally devoid of all solid elements of any kind. The three filtered diphtheritic infusions were severally injected into three,[7] two,[8] and again two[9] rabbits, the filtered Cohn fluid[10] and liver infusion[11] each into a single rabbit. Not the slightest sign of a local lesion or of any constitutional disturbance followed any of these injections, while in all cases the unfiltered infusions were found upon injection to be more or less virulent.

These various experiments thus seemed to demonstrate beyond reasonable doubt that the infectious element in diphtheritic membrane, putrid Cohn fluid, and the like, is separable by filtration from an aqueous infusion. And when the filtration is thorough, as by means of the clay filter, the removal of all noxious principles from the infusion is apparently absolute. From this fact the inference may be fairly drawn that the infectious principle or principles is

1 Dr. John S. Billings, U. S. Army.
2 Experiments 110–113. Part II., p. 704.
3 Experiments 106–109. Part II., pp. 703, 704.
4 Experiment 114. Part II., p. 705.
5 Experiments 116, 117.
6 Cases VII., VIII., and IX.
7 Experiments 28–30.
8 Experiments 37, 38.
9 Experiments 42, 43.
10 Experiment 101.
11 Experiment 115.

resident in some solid element or elements of the gross material, and cannot be separated therefrom to any recognizable extent by the action of cold water.

This being assumed to be true, the next important point for differentiation seemed to be to determine if the noxious principle be specially connected with the *bacteria* found in the infectious materials, and if it be co-extensive with the number and with the degree of vital activity of these organisms. The great practical importance of the question thus raised is obvious. For if, as has already been claimed,[1] the poison resides wholly in these organisms, and infection of the human system with diphtheria occurs only by bodily entrance of living bacteria into the blood, the whole current of preventive treatment will be turned to devising means for either destroying the vitality of such poison-bearing bacteria, or to prevent their passage through the tissue of the mucous membranes into the blood-vessels. In attacking this question by experimentation it was plain that a roundabout method must be adopted, since the direct plan of bodily separation of the bacteria from the other solid elements of the infectious material was obviously impossible. Hence the procedure that seemed to us most promising, was to treat a piece of diphtheritic membrane in such manner as to destroy as far as possible the vital activity of the contained bacteria, without otherwise affecting the elements of the membrane, and then observe the comparative effects of inoculation of the materials so treated on the one hand, and of the original pure membrane on the other. For carrying out the projected treatment, the obvious procedure of boiling the material was deemed improper, for, though by this means the bacteria would doubtless be killed, yet at the same time all albuminous matter would be permanently altered by coagulation. Failure to infect, on the part of boiled matter, might therefore with equal likelihood be assigned to the coagulation of an albuminous chemical poison, as to the killing of an assumed infecting living organism. For a similar reason, in resorting to the only other possible procedure, namely, impregnating the material with some agent fatal to bacterial life, all substances chemically disorganizing to animal matters were debarred from use. For an obvious reason furthermore it was necessary that the agent used should, itself, when inoculated, be practically free from all poisonous and even irritant effect. From all these considerations it seemed to us likely that the necessary conditions would be best fulfilled by impregnating the infectious matter with *Salicylic acid*, for we would seem to have in this substance one at once non-poisonous, slightly irritant only, with as little chemical action on animal products as could be expected, and yet with very great power to arrest more or less permanently the vital activity of bacteria and allied organisms. We accordingly determined to use this substance for the purpose described. But inasmuch as at the time of instituting these experiments (now nearly two years ago), much less was known than now of the properties of salicylic acid in its relation to living forms and substances, we deemed an investigation into these properties a necessary preliminary to the proposed inoculation experiments. We accordingly instituted two sets of experiments, one to determine the effect of salicylic acid upon the life and health of the rabbit, and the other to test exactly the degree of its power over the vital activity of bacteria. For the former purpose we inoculated a paste of salicylic acid and water into the muscular tissue of the thighs of three rabbits,[2] in the usual manner. No effect whatever followed, either constitutional or local, except a limited disintegration of the tissue impregnated with the acid.[3] But even here there was no reaction in the surrounding tissues, nor the slightest appearance of hyperæmia or infiltration of leucocytes. Next, to test more thoroughly the possible poisonous effect of the acid after absorption, we injected hypodermically salicylic acid dissolved in an aqueous solution of sodic

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[1] Oertel.

[2] Experiments 125–127. Part II., p. 707.

[3] See Experiment 125. Part II., p. 707.

phosphate.[1] A litter of young rabbits weighing 1¾ pounds apiece was used in the experimentation, and setting aside a death where the hypodermic needle accidentally pierced the liver, the result was that the animals which received five, ten, and fifteen grains, respectively, of salicylic acid, survived, but one which received twenty-four grains died in about twenty minutes. But in order to dissolve the twenty-four grain dose of the acid, seventy-two grains of sodic phosphate and nearly three fluid drachms of water were required, and to administer this bulk of fluid ten separate injections, of a syringeful each, had to be practiced one after the other at different points upon the back and belly of the animal. Conceiving therefore that the death after administration of this dose might be due, not to the salicylic acid, but either to the sodic salt or the physical violence of the repeated injections, the operation was repeated on another animal with a simple sodic solution, containing no salicylic acid;[2] death followed as in the former instance. From all these facts therefore there seemed no further question that in the comparatively small quantities in which it was intended to be used, salicylic acid was practically harmless to the rabbit. To test next the power of the acid over the vital activity of bacteria, test-tubes containing various putrefiable solutions were charged with graduated percentages of salicylic acid, set aside in a warm place and watched for the first beginning of turbidity, which, as is well known, is always a sign of beginning bacterial development. The results were as follows: In the first series of experiments Cohn's fluid was used,[3] and it was found that when the charge of salicylic acid was less than 1 part to 2,500 or 3,000, the solution became turbid almost as fast as plain Cohn's fluid, to which none of the acid had been added, namely, in three or four days. Where the charge was from 1 to 2,000 or 3,000 (the results varying slightly with different samples of the salicylic acid), clouding was postponed for several days later, and charges of 1 to 1,500 and upward kept the fluid perfectly clear during the twelve days that the observation of this series lasted. In another series human urine which had been boiled and filtered was used,[4] with the result that a charge of 1 to 2,500 was necessary to effect any postponement of the normal period of beginning turbidity, and that a charge of 1 to 1,000 was the lowest that kept the urine clear during the eight days of the observation. In another series where the observation was more prolonged a weak meat decoction already inoculated with living bacteria was employed.[5] This was made by first preparing the graduated solutions of salicylic acid in distilled water (the solution being effected by addition of a little sodic phosphate), and then inoculating a fluid drachm of each solution with a single large drop of a strong decoction of meat in which bacteria were already swarming. The results were as follows: Test-tubes charged respectively with 1 part of salicylic acid to 100 and 250 remained clear during the whole seventy-eight days of observation. Those containing 1 to 500 and 750 were clear on the twenty-eighth day, but were found cloudy when next examined on the seventy-fourth day. A charge of 1 to 1,000 postponed the clouding twenty-eight days, but charges of 1 to 2,500 and less had scarcely any influence. The general results of these experiments then was that even in practically minute proportion the addition of salicylic acid to putrefiable solutions prevents and suspends bacterial development.

These various experiments thus seem to show that in salicylic acid we had an available material for our present purpose, namely, a substance practically harmless to the rabbit, of little chemical action upon animal matter, yet of great power to suspend or prevent the vital activity of bacteria. We accordingly proceeded to institute comparative observations between inoculations of pure diphtheritic membrane and tongue-scrapings on the one hand,

[1] Experiments 128–133. Part II., pp. 707, 708.
[2] Experiment 137. Part II., p. 709.
[3] Experiments 139–175. Part II., p. 710.
[4] Experiments 176–186. Part II., p. 712.
[5] Experiments 187–206. Part II., p. 712.

and on the other, of the same substances thoroughly triturated before inoculation with salicylic acid. The experiments were as follows: In one case,[1] fourteen grains weight of diphtheritic membrane was first triturated with fourteen minims of water containing two per cent. of salicylic acid, and six per cent. of sodic phosphate in solution. Next, five grains of solid salicylic acid was added, and the whole thoroughly triturated in a mortar for half an hour. The pulpy mass thus formed was then inoculated in the usual way upon three rabbits.[2] All developed the usual lumps at the point of inoculation, but the lesions were not extensive, and the general health of the animal did not seem to suffer. One animal was accidentally killed a month after the inoculation, but the other two survived. The same membrane without the addition of salicylic acid was also inoculated upon two[3] rabbits, as a control experiment. One of these animals was found dead on the second day, and the other on the third, but in the latter case, the animal was found partly out of the cage, between the bars, and the death may have been caused by injury. In both there was diffuse inflammation with the usual pasty infiltration at the sites of the inoculation, but the lesions were not severe. In another case,[4] a piece of a different specimen of diphtheritic membrane was treated with salicylic acid in the same way as the foregoing, and inoculated upon three rabbits.[5] One animal was found dead on the fourth day, and examination showed the usual pasty infiltrations, although there was not much circumjacent inflammation. The second died on the fifteenth day, much emaciated, with large lumps of the usual character, and about the average degree of inflammation of surrounding parts. The third animal survived, but also developed large lumps which ulcerated and discharged a white creamy matter, almost devoid of smell. The same membrane, pure, was inoculated upon three rabbits[6] for comparison. All three developed lumps which ulcerated and discharged *offensive* matter. Two survived, one becoming somewhat thin and mangy, but subsequently recovering; and the third died on the twenty-third day, much emaciated, and with abscesses at the sites of inoculation. Analogous experiments with tongue-scrapings were as follows: Six grains weight of pulpy tongue-scrapings from a furred tongue, three hours after removal, was rubbed thoroughly with one grain of salicylic acid, and inoculated upon three rabbits.[7] The first animal presented on the sixth day an extremely emaciated and dull appearance, and had thick lumps in each thigh. These were pricked and the matter examined for bacteria, but only a few motionless rod-forms could be found. The animal died the next day. Rabbit No. 2 died on the sixteenth day, and post-mortem examination showed an extensive patch of yellow material at each inoculation site, with, however, no signs of circumjacent irritation. No living bacteria, and but very few motionless ones were to be found in the matter composing the patches. The third rabbit survived, and never lost his fine general condition, but large lumps, attaining on one side the size of a pigeon's egg, formed at the inoculated spots. The matter composing these was examined on the seventh and again on the twenty-fifth day, but no bacteria could be found on either occasion. These experiments were repeated with another specimen of tongue-scrapings, as follows: Some of the mixed scrapings of five successive mornings from a furred tongue was treated with salicylic acid solution, and then with the solid acid exactly as were the diphtheritic membranes in the experiments with the same last described. And it was an interesting and suggestive observation that, whereas the mixed scrapings before this treatment had, from their staleness, a horribly offensive odor, all smell disappeared after the

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[1] Case XIII. Part II., p. 690.

[2] Experiments 64–66.

[3] Experiments 62, 63. Part II., p. 691.

[4] Case XIV. Part II., p. 692.

[5] Experiments 70–72. Part II., p. 693.

[6] Experiments 67–69. Part II., pp. 692, 693.

[7] Experiments 80–82. Part II., p. 697.

salicylic acid was thoroughly incorporated with the mass. Six rabbits[1] were now inoculated with the acid-treated mixture. All developed lumps, but not very large; two died, one on the twelfth, and one on the nineteenth day, the other four survived. Some of the same scrapings, not treated with the salicylic acid, was inoculated into two other rabbits.[2] Both animals were affected about as those last described, and one died on the sixth day; but as this was a young rabbit, and others of the same litter died about the same time without having been experimented on at all, it is doubtful if the present death were due to the effects of the inoculation.

Reviewing, now, these experiments with material treated with salicylic acid, it will be seen that the original matters, both the specimens of diphtheritic membrane and of tongue-scrapings—varied a good deal in virulence, but that such poisonous property as they had, was scarcely affected by the thorough trituration with the acid. The only obvious effect of the salicylic acid treatment seemed to be to prevent to a certain extent the matter composing the lumps from becoming offensive, and to prevent much multiplication of bacteria in the same.

Although not bearing directly upon the matter under investigation, yet it seemed to us important to know if other and specific animal infectious matter would be equally unaffected by admixture with salicylic acid. Accordingly it occurred to us to mix vaccine lymph with a solution of salicylic acid, and then see if after such treatment the lymph would preserve its activity. In pursuance of this idea we requested Dr. Frank P. Foster to allow some bovine vaccinations to be made at his establishment after the manner indicated. He kindly consented, and vaccinations were accordingly made by Dr. W. F. Cushman and Mr. H. Lawrence, under direction of one of us (T. E. S.), as follows: Salicylic acid was dissolved in water by the help of sodic phosphate, two solutions being made, one containing the acid in the proportion of 1 to 250, and one in the proportion of 1 to 500. All being ready for the vaccination, some of the lymph to be used was mixed with each of these solutions, measure for measure, and the mixture allowed to stand for one minute before inoculation. Then both the pure lymph and the mixtures were inoculated upon the same animal.[3] The result was that the admixture of the salicylic acid had not the slightest effect upon the virulence of the lymph, just as perfect pocks forming where the mixtures had been inoculated as where the pure lymph had been used. These experiments then, so far as they went, were in harmony with those preceding.

So far, now, as regards the inoculations on the rabbit of the salicylic-treated diphtheritic membrane and tongue-scrapings, we have the fact that an admixture of the acid in overwhelming quantity (overwhelming from the point of view of its power to suspend the vital activity of bacteria), was yet almost powerless to affect the poisonous property of the matter subjected to treatment. Hence the important conclusion suggests itself that this same quality of infectiousness in the diphtheritic membrane and tongue-scrapings must be independent of the vital condition of the bacteria present in the matter.

In this connection it becomes an interesting question to review the histories of the cases from which the membranes used for inoculation were drawn, and see what relation appears between facts connected with the disease in the human subject and the disease produced in the inoculated animals. In this analysis, cases I. and II. must be disregarded, because no inoculations were made with the material derived from them upon the *muscle* of the rabbit, and there is, therefore, no means of rating the comparative virulence of such material with the membranes from the other cases which were all used for muscle-inoculations. Taking, then, the

[1] Experiments 85–90. Part II., pp. 698, 699.

[2] Experiments 83, 84. Part II., p. 698.

[3] Experiment 138. Part II., p. 710.

other twelve cases, and studying the results of inoculation of the solid membrane or unfiltered aqueous infusion of the same in the thighs of rabbits, the membranes may be rated as follows in order of inoculable virulence, beginning with the most active: V., X., VIII., III., XIII., VI., VII., XI., IX., XIV., XII., IV.

Studying, now, the cases in this order the following points become manifest: I. *There is no relation between inoculable virulence of a membrane and the time after detachment that has elapsed before the material is used for inoculation.* For, of the two membranes heading the list for virulence, one (case V.) was used on the very day of detachment, and the other (case X.) not until three days old and already beginning to putrefy. On the other hand the membrane that proved the least virulent of all (case IV.) was used the day after detachment, and among the feebly virulent (case XIV.) the membrane was three days old. II. *There is no relation between inoculable virulence of a membane and the quantity of bacteria present in the same.* For, bacteria were abundant among both the most and least virulent of the membranes, and on the other hand the membrane third on the list for virulence (case VIII.) contained very few bacteria. III. *There is a rough relation between inoculable virulence on the part of the membrane and the severity of the original disease in the human subject, so far as this can be estimated by the issue of the case in death or recovery.* For, taking the cases in the order of inoculable virulence of the membranes as given above, the terminations of the cases in death or recovery read thus: *Death*, *recovery*, *death*, *death*, *death*, *recovery*, *death*, *death*, *unknown*, *recovery*, *recovery*, *recovery*.

Besides the foregoing work, we had proposed for ourselves many other experiments and observations, notably the making of careful post-mortem examinations on human subjects dead of diphtheria, and the sanitary inspection of houses where many cases of the disease had occurred. Whenever opportunity offered we followed out our intentions in these regards, but during the period covered by the investigations these opportunities, in spite of our best efforts, were so few, that the desultory observations made are not numerous enough to generalize from. Other laboratory experiments were also planned and begun. These looked to the treatment of infectious matters by various chemical and physical agents, with a view of determining the nature of the poisonous principle by observing how the infectious quality might be affected by such treatment. But the investigations had to be brought to a close before enough of these later experiments had been made to draw conclusions from.

The results of our investigations may therefore be summed up as follows:

I. Inoculation of diphtheritic membrane into the muscular tissue of the rabbit produces severe local lesions, and even constitutional disturbance and death. But these effects differ so in their pathology and clinical history from diphtheria in the human subject, that there is no warrant for defining them as diphtheria, or for applying conclusions drawn from observation of this inoculation-disease in the rabbit to the case of diphtheria in man.

II. Effects exactly similar to the foregoing and of equal severity can moreover be produced by inoculation of a material not only non-diphtheritic, but non-infectious to the human subject under conditions where diphtheritic membrane *is* infectious, *i. e.*, when brought into contact with the mucous membrane of the mouth and throat. The material referred to is the pulpy scraping of the upper surface of the healthy human tongue.

III. Effects generally similar to the foregoing, though not of equal intensity, can furthermore be produced by inoculation of a putrescent matter which is not even of immediate animal origin, namely Cohn's fluid, allowed to spontaneously decompose. (Cohn's fluid is

simply an aqueous solution of ammonic tartrate, potassic and calcic phosphates and magnesic sulphate.)

IV. The foregoing inoculation effects are not due to simple mechanical irritation, for inoculations of sand produce no effect whatever.

V. Thorough filtration of a proven virulent aqueous infusion of diphtheritic membrane or of putrid Cohn's fluid removes the infectious property of the same. Hence in such diphtheritic infusion the poisonous quality probably inheres in some *particulate* thing, from which it is not separable by the action of cold water.

VI. Thorough trituration of proven virulent diphtheritic membrane and tongue-scrapings with a high percentage of salicylic acid fails not only to remove, but even markedly to modify, the intensity of the infectious quality of those substances. Hence, since salicylic acid in even a minute percentage is capable of permanently suspending the vital activity of bacteria, the inference is that the infectious quality of diphtheritic membrane upon the system of the rabbit is not correlated to the vital activity of the bacteria present in such membrane.

VII. If, as is not improbable, the noxious principle in diphtheritic membrane which produces in rabbits the effects described, be the same with or even analogous to the principle which produces diphtheria in man by direct infection, then the conclusion of VI. will apply to the infectious quality of such membrane in its relation to the reproduction of diphtheria in the human subject. If this be the case, it follows as an important practical corollary that *there is no theoretical ground for assuming that preventing the bacteria of a diphtheritic patch from making their way through the underlying mucous membrane will, per se, prevent general diphtheritic infection of the system.*

VIII. There is no relation between inoculable virulence of a diphtheritic membrane and the period, within three days, that has elapsed between the detachment of the membrane and the inoculation with the same, nor between inoculable virulence and gross amount of bacteria present in the membrane.

IX. There is a rough relation between inoculable virulence of a diphtheritic membrane and the severity of the original case of diphtheria, so far as this can be estimated by the termination of the case in death or recovery.

But it must be distinctly understood that these nine propositions are not put forth as *proven*, but merely as the results of our experiments and observations so far as the latter go, stated in abstract form. Before the propositions can be considered proved as truths, a large number of corroborative experiments will have to be made.

PART II.—RECORD OF EXPERIMENTS.

I.—INOCULATIONS WITH DIPHTHERITIC MATTER.

Case I.

March 29, 1875, received from Dr. Harwood Wakeman a piece of diphtheritic membrane, wrapped in damp cotton, and this encased in rubber tissue. Accompanying was the following memorandum: "Fresh at 4 P. M.; has been pretty well soaked with an application to the

throat of the child of diluted muriatic acid; child four years old; sixth day since first appearance of membrane." Not stated whether removed from living throat or post-mortem. The specimen, on soaking a bit in glycerine, and examining microscopically, showed a general infiltration with minute punctate bodies over the surface, and, imbedded in the substance, spheroid collections entirely composed of such bodies closely packed (nests or balls of bacteria).

Twenty-four hours after removal the following inoculations were made, the animals being young rabbits of the same litter.

a. Inoculation of cornea with pure membrane.

Experiment 1:

March 29, 1875. Right cornea of a rabbit pricked and scratched in many places with a clean needle, and a piece of diphtheritic membrane thoroughly rubbed into the wounds. Piece finally left in cul de sac of upper lid. Left eye similarly scratched, but no inoculation made.

March 30. Left eye, punctures healing, without irritation. Right eye, lids glued with catarrhal secretion, forming thick crusts on the outside. On opening lids, points of puncture cloudy and elevated. General catarrhal conjunctivitis, but no thickening of the conjunctiva nor appearance of a diphtheritic patch.

March 31. Left eye, punctures healing. Right eye, catarrhal secretion less, but gray points of infiltration at punctures are perhaps slightly more distinct, with some general corneal haziness in that neighborhood. Animal feeds well, and is lively.

April 1. All the morbid symptoms subsiding.

b. Inoculation of cornea with aqueous infusion of membrane.

Experiment 2:

March 29, 1875. A small piece of membrane was thoroughly triturated with ℥ ij of cold water, and steeped in the same for half an hour. Both corneæ of a rabbit were then inoculated with the infusion in the manner described above.

March 30. Right eye, punctures apparently healing, without irritation. Left eye, points of puncture cloudy; general conjunctivitis, but less than in Experiment 1.

March 31. Right eye healing. Left eye, general improvement.

c. Inoculation of cornea with aqueous infusion of membrane, boiled.

Experiment 3:

March 29, 1875. A little of the foregoing infusion was boiled in a test tube for five minutes, and then allowed to cool. Both corneæ of a rabbit were inoculated with the same, when cold.

March 30. Both eyes healing, without irritation.

March 31. Both eyes nearly well.

CASE II.

April 4, 1875, received from Dr. J. Lewis Smith the trachea, bronchi, spleen and kidneys of a child supposed to have died of diphtheria twenty-four hours previous. The tissues seemed

well preserved and had no bad smell. The mucous membrane of the trachea and bronchi was reddened, but there was no appearance of a membrane. The child from whom the specimens were taken had a moderate inflammation of the glands of the neck. On the third day there was a catarrhal discharge from the nostrils, and an epistaxis. A trace of albumen appeared in the urine, and the child died on the sixth day.

a. Inoculation of cornea and conjunctiva with reddened mucous membrane of trachea.

Experiment 4:

April 4, 1875. Inoculated right cornea of a rabbit, with the scrapings of the reddened trachea; in left eye, a piece of the conjunctiva was snipped out, and a piece of the mucous membrane inserted in the wound.

April 5. Right eye, hardly any reaction. Left eye, considerable conjunctival inflammation.

April 6. Right eye almost well; left eye, conjunctivitis diminishing, but the piece of mucous membrane is still in situ, and appears to be firmly imbedded in the conjunctiva. No constitutional disturbance.

April 7. Right eye well; left eye, conjunctivitis subsiding; piece still in situ; rabbit doing well.

b. Inoculation of cornea with kidney-tissue.

Experiment 5:

April 4, 1875. Right cornea of a rabbit inoculated with a piece of the kidney.

April 5. A good deal of keratitis.

April 6. Keratitis increased. Rabbit does not feed as well as usual. The keratitis is limited to a small area, ¼ by ⅛ inch, and the cornea is quite opaque.

April 7. Rabbit eating better. Corneal redness diminishing, opacity as before. Removed with forceps as much matter as could be gotten off the cornea, some of the epithelium coming off with it. There was a slight layer of fibrine on the surface, and the epithelial cells contained numbers of brownish granules.

April 8. No increase in inflammation. Conjunctivitis and keratitis subsiding. Opacity as before. Rabbit's condition good.

April 10. Rabbit well.

Case III.

April 13, 1875, received from Dr. Horace T. Hanks a piece of diphtheritic membrane of soft consistence and grayish yellow color, taken from the tonsils of a girl four years old. About April 1, patient was taken with loss of appetite and high fever. April 4, she was first seen by Dr. H. and a membrane was then observed on the tonsils—later it appeared in the nares. It became detached on the sixth day of the disease, but symptoms of blood poisoning set in and the child died on the seventeenth day. There had been no obstruction of the larynx or paralysis.

a. Inoculation of cornea with pure membrane.

Experiment 6:

April 13, 1875. Both corncæ of a rabbit inoculated with the pure diphtheritic membrane.

April 14. Slight keratitis and conjunctivitis only.

April 15. Eyes improving.

April 17. Eyes nearly well.

Experiment 7:

April 13, 1875. Same procedure as foregoing.

April 14, 15, 17. Results same as in foregoing.

b. Inoculation of muscular tissue with pure membrane.

Experiment 8:

April 13, 1875. Muscular tissue of both thighs of a rabbit inoculated with pieces of pure membrane. (For method of these and all subsequent inoculations into the muscular tissue, see ante, Part I.)

April 14. No reaction of any moment.

April 15. Died about forty-five hours after the inoculation. *Autopsy* twenty-seven hours later: Serous infiltration of the subcutaneous connective tissue over the thighs as far back as the lumbar vertebræ; isolated patches of the same over the abdomen as high as the foreshoulder. *Right thigh:* Both the aponeurosis and muscular substance of the entire thigh closely stippled with small ecchymoses. On vertical section through inoculation site, find a grayish-white patch about equal in size to the original inoculated material; this, under the microscope, is found to be composed of cellular bodies undergoing fatty degeneration, much free granular matter and a few bacteria. Around the muscle wound the tissues were reddened and ecchymosed, and there was much serum in the intermuscular connective tissue. The muscle-substance adjoining the wound when compared with that of a *non*-inoculated rabbit that died at about the same time was found in no wise different, both were undergoing post-mortem changes; the striæ were still well marked, and a few bacteria were found in both cases, but most numerous in the tissue from the non-inoculated animal. Considerable infiltration of the muscular fibres with fine oil-drops became apparent after three hours soaking in glycerine. The appearances in the *left thigh* were in all respects similar to those in the right. The *blood* drawn from the right ventricle of the heart contained a few small rod-bacteria, but far fewer than the blood of the non-inoculated rabbit already mentioned. The *liver* contained small echinococcus cysts. The *kidneys* were carefully examined for bacteria, fresh, and after soaking pieces in caustic potassa. No bacteria were found nor any appearances different from what obtained in the kidney of the non-inoculated rabbit.

c. Inoculation of cornea with aqueous infusion of diphtheritic membrane.

Experiment 9:

April 13, 1875. Both corneæ of a rabbit were inoculated with an aqueous infusion of the membrane.

April 14. No result of any consequence.

April 15. Same condition.

April 17. Nearly well.

d. Inoculation of muscular tissue with boiled, aqueous infusion of diphtheritic membrane.

Experiment 10:

April 13, 1875. Similar procedure to foregoing with the infusion after boiling and cooling.

April 14, 15, 16. Results as in foregoing.

e. Inoculation of muscular tissue with filtered aqueous infusion of diphtheritic membrane.

Experiment 11:

April 13, 1875. Thigh of rabbit inoculated with the unboiled infusion after filtering through one thickness of filtering paper.

April 14, 15, and 17. Results as in foregoing.

Case IV.

April 14, 1875, received from Dr. Horace T. Hanks another piece of membrane taken the night before from the nares of a girl three years old. The child was taken with an acute nasal catarrh, and on the second or third day this membrane formed in the nares. It became detached a day or two later and the child recovered in a week without having had any alarming symptoms. The specimen was not firm and tough like genuine diphtheritic membrane, but soft and glutinous. Under the microscope it was seen to be composed of leucocytes and granular matter held together by tough mucus.

a. Inoculation of cornea and muscular tissue with pure membrane.

Experiment 12:

April 14, 1875. Both corneæ and one thigh of a rabbit were inoculated with the pure membrane. No obvious effect was produced.

Experiment 13:

April 14, 1875. Same procedure and same result as foregoing.

b. Inoculation of cornea and muscular tissue with aqueous infusion of pure membrane.

Experiment 14:

April 14, 1875. Both corneæ and one thigh of a rabbit were inoculated with an aqueous infusion made by triturating in one fluid ounce of water a piece of the membrane about $\frac{1}{8}$-inch square. No recognizable effect followed.

c. Inoculation of cornea and muscular tissue with aqueous infusion of membrane, boiled.

Experiment 15:

April 14, 1875. Both corneæ and one thigh were inoculated with the foregoing infusion after it had been boiled for three minutes. No recognizable effect followed.

d. Inoculation of cornea and muscular tissue with aqueous infusion of membrane, filtered.

Experiment 16:

April 14, 1875. Both corneæ and one thigh were inoculated with the infusion of "*b*" after filtration through a single thickness of filtering paper. Death forty-five hours later. *Autopsy:* Small whitish spot in the muscular substance at the site of the thigh inoculation; striæ obliterated in the circumjacent muscular fibres, and numerous rows of fat globules developed. After soaking the fibres in 10 per cent. caustic potassa for twenty-four hours, the sarcous matter and fat globules both wholly disappeared. Nothing abnormal to be found in any other part of the body. Corneæ entirely healed. (N B.—This animal was one of a litter of young rabbits, others of which, not subjected to experimentation, died at about the same time.)

Case V.

April 23, 1875, received from Dr. George W. Rachel a piece of diphtheritic membrane removed the same day, and wrapped in moist rag and enveloped in rubber tissue. The case was that of a boy three and a half years old, whom Dr. Rachel first saw on the third day of his illness. The fauces and nasal cavity were then almost wholly covered by a membrane, and the urine was albuminous. Difficulty of breathing began on the seventh day and continued till death, which took place four days later. The patient became finally comatose, and the temperature reached as high a figure as 106.25.° Others in the same family were attacked by the disease, and there were other cases still in the neighborhood. The specimen of membrane as received was apparently in excellent preservation; it contained bacteria.

a. Inoculation of muscular tissue with pure membrane.

Experiment 17:

April 23, 1875. Both thighs of a rabbit inoculated with the pure membrane.

April 25. Dead forty-five hours after inoculation. *Autopsy.—Left thigh:* Skin wound closed, but attached to the inner surface of the skin at this point was a small yellowish mass about the size of a No. 8 bird-shot. At the point of inoculation in the muscle was a yellowish mass spread out beneath the muscle sheath. The muscular tissue itself was mottled red and white, but there was no extensive inflammatory change in the adjacent parts. *Right thigh:* Skin wound closed. Over the whole hinder half of the back and belly the subcutaneous tissue was infiltrated with serum, and unnaturately adherent to the skin and sheaths of the muscles. The muscles and their sheaths were stippled with numerous ecchymoses. Track between the skin and muscle-wounds was filled with a yellowish pasty material. This, under the microscope, was found to consist of the elements of connective tissue stuffed with leucocytes with a little granular matter. No recognizable bacteria. The same matter from the right thigh was essentially similar in composition. *Blood* from the right ventricle contained numbers of rod shaped bacteria. *Trachea* showed post-mortem congestion, but no signs of a membrane. *Kidneys* normal; no signs of bacteria.

Experiment 18:

April 23, 1875. Both thighs inoculated with pure membrane, as in the foregoing experiment.

April 25. Death between thirty and forty hours after inoculation. *Autopsy.—Left thigh:* Skin wound closed. At point of inoculation was a yellowish white mass, partly upon the surface of the muscle, and partly infiltrating the substance of the same, the overlying tissues adherent to one another and to the skin. The muscle itself, at the point of inoculation, was not much affected, but further away it was spotted with ecchymoses, and there was a large ecchymosis, one-half inch in diameter, on the back. *Right thigh:* Appearances generally similar, but less inflammation in the connective tissue, though more in the muscular. *Blood* from the right heart full of rod-bacteria, and the corpuscles much changed. Many of them had become adherent, forming irregular masses. *Kidneys* not congested. *Trachea:* Unnaturally red, but no membrane had formed. Microscopically, the yellow material from the sites of inoculation consisted of connective tissue stuffed with leucocytes and granular matter.

Experiment 19:

April 23, 1875. Both thighs inoculated with pure membrane, as in the two foregoing experiments.

April 25. Dead between thirty and forty hours after inoculation. *Autopsy:* Skin-wounds closed on both sides. Subcutaneous connective tissue of thighs much thickened, and of a mottled yellow, red and white color. Small collections of yellow material at points of inoculation, and the adjacent muscular tissue much reddened. Appearances generally similar to those in Experiment 17.

Case VI.

May 2, 1875, received again from Dr. George W. Rachel a piece of diphtheritic membrane. The patient was a girl, two years old, sister to the patient of Case V. She was taken with vomiting, fever, and drowsiness, and on the following day, membrane formed upon the left tonsil and pharyngeal wall. It was detached the same day by forceps. No albumen in the urine, larynx not obstructed, recovery after four days' illness. The specimen was several days old when received. It was found to be made up of a meshwork of the mycelium of a fungus stuffed with masses or "balls" of closely-packed bacteria, comparatively few leucocytes, a little fibrin, and a considerable quantity of large fat globules. After treatment with an alkali, the balls of seemingly spherical bacteria disappeared, and the field became crowded with rod-forms, free.

a. Inoculation of muscular tissue with pure membrane.

Experiment 20:

May 2, 1875. Both thighs of rabbit inoculated with pure membrane.

May 3. Animal apparently well in the morning, but towards evening became languid and refused food.

May 4. Found dead. *Autopsy*, at least fifteen hours after death: No extensive local lesions on either side. The piece of inoculated membrane had disintegrated, its leucocytes were undergoing fatty degeneration, and the compressed bacteria-balls had disappeared, giving place to a general infiltration of free rod-bacteria. Very little disturbance in the muscular tissue at the point of inoculation. *Blood* contained an abundance of rod-bacteria and granular matter, the latter dissolving on addition of caustic potassa solution. *Kidneys* contained bacteria. (N. B.—It will be remembered that this examination was at least fifteen hours after the death of the animal.)

Experiment 21:

May 2, 1875. Both thighs inoculated with pure membrane, as in the foregoing.

May 4. A vein in the ear was pricked, and the blood examined microscopically for bacteria, but none were found.

May 19. Animal's general condition pretty good. A lump could be felt under the skin at the point of inoculation on the right side. The skin was snipped, and the parts beneath inspected. The lump was a collection of yellowish-white material about the size of a pea; it had no connection with the skin-wound, which was healed. The material of this lump was found to consist of connective tissue stuffed with leucocytes, with a few large corpuscles containing good-sized oil-drops, a very moderate number of free rod-bacteria, and some granular matter. The substance had no offensive odor.

May 23. Found dead.

May 24. *Autopsy.—Right thigh:* The lump described above had diminished in size. Its substance was of the same composition, except that the bacteria had almost wholly disappeared. No new appearances since previous examination. *Left thigh:* Long, narrow collection of yellow material in the subcutaneous connective tissue, one-half inch long by one-tenth inch wide; consists of enormous numbers of closely-packed fat-globules of various sizes, with a few corpuscles, themselves stuffed with oil-drops. No bacteria. Site of inoculation in the muscle could not be found. Skin-wound healed, and no hyperæmia or infiltration of tissues adjoining the adventitious mass. *Blood* from right and left hearts free from bacteria. *Internal organs* all apparently healthy.

Experiment 22:

May 2, 1875. Both thighs inoculated with pure membrane, as in foregoing.

May 21. Dead. *Autopsy:* Body greatly emaciated. *Left thigh:* Circular, thin layer of yellow material, ¼ inch in diameter, closely adherent to the outer surface of the aponeurosis of the muscle; material pulpy, and found to consist of leucocytes in an advanced stage of fatty degeneration, with a good deal of free oil-drops of various sizes. Beneath the aponeurosis, immediately under this layer, was a linear yellow streak about ⅛-inch long, found to be almost entirely made up of closely packed minute oil-drops of various sizes. *Right thigh:* Yellow layer similar to above, but smaller, in the deep connective-tissue. In neither of these patches were there any bacteria, nor was there on either thigh any serous infiltration or hyperæmia of any of the tissues. *Blood* contained no bacteria. *Internal organs* apparently healthy.

Experiment 23 :

May 2, 1875. Both thighs inoculated with pure membrane, as in foregoing.

May 7. Lump felt at point of inoculation on left side ; movable with the skin, and seemed to be about the size of a pea. On exposing the parts, a small, round, white, semi-solid mass, the size of a pea, was found lying in the loose subcutaneous tissue, over the thigh. The connective tissue immediately surrounding the mass was slightly thickened and reddened, forming a sort of capsule, but was not adherent to the aponeurosis of the muscle beneath. The mass was carefully dissected out, and bodily removed. No lesion of the aponeurosis of the muscle or of the muscle itself beneath the site of the tumor could be found. The material composing the tumor consisted of closely packed leucocytes undergoing fatty degeneration, free granular matter, free fat-globules, and a moderate number of rod-bacteria in active motion. No "ball" or "nests" of bacteria.

May 24. Animal somewhat poorly, but no lumps.

July 10. Animal still living, but in poor condition.

Experiment 24 :

May 2, 1875. Right thigh only inoculated with pure membrane, as in foregoing.

June 10. Found dead. *Autopsy:* Body greatly emaciated. In inoculated side a small yellow mass $\frac{1}{4} \times \frac{1}{8}$ inch in diameter; circumjacent skin and connective tissue thickened and reddened. Tumor composed of connective-tissue elements swollen and infiltrated with oil-globules. Wound of inoculation in the muscle wholly healed. *Blood* contained a very few rod-bacteria. *Lungs* normal. *Liver* moderately fatty. *Kidneys* showed a moderate number of small bodies apparently possessed of independent motion, and possibly bacteria.

Case VII.

May 21, 1875, received from Dr. Lewis W. Oakley, of Elizabeth, N. J., a piece of diphtheritic membrane detached from the throat of a child eight years old on the sixth day of the disease. When first seen, both tonsils, the uvula and most of the soft palate were already covered by membrane. There was no albumen in the urine, no marked symptoms of laryngeal obstruction, but decided and constant signs of constitutional infection. The case terminated in death fifteen days after it was first seen by the physician. The specimen of membrane was two days old when the following inoculations were made with it :

a. Inoculation of the muscular tissue with pure membrane.

Experiment 25 :

May 21, 1875. Both thighs of a rabbit inoculated with the pure membrane.

May 22. Animal well.

June 8. Animal thin, but lively. The skin wound on the *right side* presented a small ulcer with puffy red sides. At site of inoculation a lump the size of a large pea could be felt. The skin was snipped, the yellow tumor pricked, and the exuding pasty matter examined. It consisted of degenerating leucocytes, granular débris, and

a moderate number of dumb-bell-shaped bodies in dancing motion, apparently bacteria. *Left side:* No lump felt. The skin was snipped and the parts beneath inspected. A shred of slightly thickened and yellowish connective tissue was found attached to the inner surface of the skin wound, and reaching toward the muscle. It was about a quarter of an inch long, and was found to consist of connective tissue elements stuffed with large oval sacs crowded with air-globules; no leucocytes; no bacteria.

June 13. Dead. *Autopsy.—Right thigh:* At site of inoculation, a cylindrical yellow mass connecting the skin and muscle-wounds, and ending in an enlargement the size of a hazel-nut; it consisted of a connective tissue wall, with soft, yellowish-white contents. The latter material was composed as above described, except that the bacteria had disappeared. *Left thigh:* Same appearance as at previous examination. *Lungs*, *liver*, *spleen*, and *larynx* normal. *Kidneys* rather congested. Body greatly emaciated.

Experiment 26:

May 21, 1875. Both thighs inoculated with pure membrane, as in foregoing.

May 22. Animal well.

June 8. Animal well. Both thighs presented lumps the size of a bean. That on the left thigh was pricked and the matter examined; it consisted of degenerating leucocytes and granular débris.

June 21. Found dead in the morning.

June 22. *Autopsy:* Body in good condition; fur sleek and clean. *Right thigh:* Yellow mass the size of a bean joining the skin and muscle-wounds, composed of fatty and granular leucocytes. *Left thigh:* Yellow mass extending from skin-wound into and through a thin thigh muscle, and communicating with a large lobulated yellow mass $\frac{5}{8}$-inch long by $\frac{3}{8}$-inch wide and deep, situated in the lax intra-muscular tissue. The material of this mass consisted of connective-tissue elements, densely infiltrated with fat granules, and the usual granular leucocytes. Only here and there, in the matter from either thigh, could a motionless rod-bacterium be seen.

Experiment 27:

May 21, 1875. Both thighs inoculated with pure membrane, as in foregoing.

May 22. Animal well.

May 28. Skin of right thigh was snipped and parts inspected. Yellowish-white mass connecting skin and muscle wounds. On opening, contents fluid, consisting of leucocytes, some free bacteria, and others collected into large masses.

June 8. Lump still to be felt on right side as big as a bean. Pricked, yielded yellow, pasty matter composed of closely packed leucocytes in good preservation, granular debris; few bacteria.

June 24. Animal in good condition; cord-like thickening only to be felt; not opened.

July 7. Animal in good condition.

b. Hypodermic injection of aqueous infusion of pure membrane after filtration through a porous clay cylinder.

A piece of the membrane weighing six grains was cut up fine, thoroughly triturated with two fluid drachms of distilled water, and allowed to steep for twenty-four hours. The infusion was then poured into a clay cylinder, such as come for use in galvanic batteries. The closed end of this cylinder was covered with a rubber cap, by which communication was made with an exhausting syringe. By exhausting the air by means of the syringe, the fluid was drawn through the cylinder to the extent of eleven minims. This filtrate was perfectly clear; and, examined microscopically, proved wholly free from bacteria or any kind of solid particles.

Experiment 28:

May 22, 1875. Three minims of above clear filtrate injected hypodermically into left thigh of a rabbit.

May 28. Animal in excellent condition; not the slightest sign of a lump to be felt.

June 26. Excellent condition; there has been no development of a lump.

July 10. Excellent condition; never any sign of a lump.

Experiment 29:

May 22, 1875. Four minims of above clear filtrate injected hypodermically into right thigh of a rabbit.

May 28. Excellent condition; no sign of a lump.

July 10. Excellent condition; there has been no lump.

Experiment 30:

May 22, 1875. Four minims of above clear filtrate injected hypodermically into left thigh of a rabbit.

May 28. Excellent condition; no sign of a lump.

July 4. Dying.

July 5. Dead. *Autopsy:* Not the slightest indication of there having ever been any effect produced at the site of the injection. Death probably from some other cause. Many animals died during the warm weather without having been experimented upon.

c. Hypodermic injection of unfiltered aqueous infusion of pure membrane.

The residue of the infusion used to obtain the filtrate above mentioned, was allowed to stand till the larger particles had settled. The turbid supernatant fluid, examined microscopically, was found to contain small broken fragments of diphtheritic membrane, and to be swarming with actively moving bacteria.

Experiment 31:

May 22, 1875. Four minims of the above turbid infusion injected hypodermically into left thigh of a rabbit.

May 28. Animal has been sickly. Lump the size of a pea felt at site of injection. Skin opened, and yellow tumor found adhering to the superficial fascia. It was pricked, and the pasty contents examined microscopically: found to consist of leucocytes and granular débris, with very few bacteria.

June 26. Animal in excellent condition. The lump seems to have diminished in size.

July 10. Animal in excellent condition.

Experiment 32 :

May 22, 1875. Four minims of turbid infusion injected in right thigh of rabbit.

May 28. Animal in good condition ; lump felt under skin.

June 8. Lump, size of a large pea, felt freely movable under skin at site of injection. Skin snipped ; tumor pricked, and yellow contents examined. Consisted of leucocytes in good preservation, and granular débris ; only an occasional bacterium. Lump left in place. Animal in good condition, though badly bitten by mate.

July 1. Lump increased to size of an almond-kernel. Pricked, and contents examined, consisted of corpuscles undergoing fatty and granular degeneration. No bacteria.

July 10. Animal in good condition. Lump shrunk to size of a split pea.

Experiment 33 :

May 22, 1875. Five minims of turbid infusion injected into left thigh of a rabbit.

May 28. Animal in good condition ; lump felt under skin. Skin snipped, and parts explored. A yellow patch, half an inch long, and a quarter wide, found in the superficial fascia. When cut into, it proved tenacious and elastic ; no matter could be squeezed from the cut surface. Material found to consist of connective tissue, stuffed with granular matter, a moderate number of leucocytes and cells full of oil-globules. A very limited number only of bacteria could be found.

July 1. Animal in fair condition ; has been shedding his hair. Lump felt to have shrunk considerably in size.

July 10. Animal in good condition.

Case VIII.

May 29, 1875, received from Dr. A. S. Church, a piece of diphtheritic membrane, detached from the throat of a patient, on the sixth day of the disease. The patient, a boy nine years old, was taken with a chill, followed by fever. The next day, membrane was found on the tonsils ; on the following day, on the uvula also, and the day after that, also, in the nares. The larynx did not become obstructed, but there was constitutional infection, and the patient died on the ninth day of the disease. The specimen was examined and used two days after it was received, being in the mean time kept wrapped in a damp cloth, protected by rubber tissue. It was found to consist of a fibrinous exudation containing a moderate number of leucocytes, multitudes of oil-globules, with very few bacteria.

a. Inoculation of muscular tissue with pure membrane.

Experiment 34 :

May 31, 1875. Both thighs of a rabbit inoculated with the pure membrane.

June 26. Animal in good condition. *Right thigh :* a small lump. *Left thigh :* small open abscess, discharging freely. Matter found to consist of normal pus, with only a small amount of granular matter, and an occasional bacterium.

July 8. In good condition.

Experiment 35 :

May 31, 1875. Both thighs inoculated with the pure membrane.

June 7. Animal died during forenoon. *Autopsy* at 2 P. M.—*Right side :* At site of skin-wound a scab ¾ inch in diameter closely adherent to underlying tissues. On exposing the subcutaneous tissue a flat layer of yellow material was found reaching half down the thigh, backwards over the rump, and upwards to the median line of the back where it met a similar exudation from the opposite side. This plaque was quite closely adherent to the skin. The muscle at the site of inoculation was gangrenous over an area ½ inch by ⅛. Adjacent to this area the muscle was mottled red and yellow, and at one point a sharply defined yellow line of demarcation separated the diseased from the healthy muscular tissue. Microscopic examination showed the material of the yellow plaque to consist of a fibrinous meshwork, containing numerous deformed corpuscles about the size of leucocytes and a large quantity of granular matter. The yellow spots in the muscular tissue contained oil-globules and smaller granules strung together, so as to give the appearance of dumb-bells or chains. *Liver* large and containing echinococcus cysts. *Kidneys* swollen, soft ; tube-cells inclined to be fatty. In the débris were similar chains and figures-of-eight to those seen in the muscular tissue. *Blood* contained a few of the same elements. *Lungs* normal.

Experiment 36:

May 31, 1875. Both thighs inoculated with pure membrane.

June 4. Dead 96 hours after inoculation. *Autopsy.—Left thigh :* Yellow patch ¼ inch in diameter in subcutaneous connective tissue. Surrounding this, a white streak extending to the median line of the back. Beyond this again, a general reddening of the tissues for the space of half an inch, and considerable serous infiltration. Thigh itself, mottled with red spots. Material of yellow patch found to consist of fibrous network holding leucocytes, abundance of granular matter and a few motionless bacteria. What looked like balls of bacteria found in the blood vessels in the muscular tissue at the site of inoculation. *Right thigh :* Small yellow patch overlying muscle-wound, with surrounding serous infiltration of the subcutaneous connective tissue. The muscle-wound was lined with a dirty, friable, yellowish-white material, and the muscular tissue below was transformed into a soft, cheesy matter, found to consist of muscular fibres in advanced fatty degeneration, with here and there a motionless bacterium. For an area an inch in diameter around this affected spot the muscular tissue was reddened. *Liver* and *kidneys* rather red and swollen. *Blood* contained no bacteria.

b. Hypodermic injection of aqueous infusion of pure membrane after filtration through porous clay.

A piece of the membrane was triturated with water, infused for twenty-four hours and then filtered through a porous clay cylinder in the manner described in "*b*" case VII. (see ante, p. 681). The filtrate was perfectly clear and free from solid elements.

Experiment 37 :

June 1, 1875. Five minims of the clear filtrate injected into each thigh of a rabbit.

June 5. Animal in excellent health and fat. Skin snipped at site of each injection and parts beneath thoroughly explored. Not the slightest sign of any lesion, nor even hyperæmia.

June 8. Died during the night. *Autopsy:* No lesion whatever discoverable. Was a young animal, and death was probably unconnected with the experimentation.

Experiment 38:

June 1, 1875. Six minims of the clear filtrate injected into each thigh of a rabbit.

June 5. Animal in excellent condition. Examination made as in foregoing, but nothing whatever found.

June 11. Animal got out of his cage and fell a considerable height, breaking his leg.

June 12. Died about noon. *Autopsy* two hours later: Body in excellent condition. No lesions whatever to be found at sites of the injections. *Larynx*, *lungs*, *liver*, normal. *Blood* from right heart contains no bacteria. Death was probably due to injury from the fall.

c. Hypodermic injection of unfiltered aqueous infusion of pure membrane.

The residue of the infusion used to obtain the filtrate of "*b*" was allowed to stand till the larger particles settled; the turbid supernatant fluid was then taken for the injection.

Experiment 39:

June 1, 1875. Six minims of the turbid infusion injected into the right thigh, and eight into the left, of a rabbit.

June 3. Died in convulsions at 3.30 P. M., about forty-eight hours after the injection. *Autopsy* half an hour later.—*Right thigh:* General serous infiltration of connective tissue, area of thickened connective tissue two inches in diameter, inclosing a yellow patch ⅝ by ¾ of an inch in area. Interior of this patch was semi-fluid yellow matter made up of granular matter, and great numbers of dumb-bell and rod-shaped bodies looking like degenerate bacteria, but not in motion. Underneath this patch the aponeurosis of the muscle was cloudy and thickened, but the muscle itself was not affected. *Left thigh:* No patch, but intense congestion of the tissues over the thigh and of the muscle itself to the depth of ⅛ inch with great serous infiltration of the subcutaneous connective tissue. *Liver* large, smooth, and congested. *Kidneys* congested. *Lungs*, *larynx*, and *heart*, normal. *Blood* from right ventricle contains no bacteria.

Experiment 40:

June 1, 1875. Six minims of the turbid infusion injected into each thigh of a rabbit.

June 4. Dead. *Autopsy:* Subcutaneous connective tissue over whole aspect of both thighs intensely congested, and skin unnaturally adherent. Where the injection had been delivered the connective tissue was infiltrated with specks of yellow material, found to be composed of débris of corpuscles and innumerable ordinary rod-bacteria. The appearance suggested that these specks were the solid portions of the original infusion as injected. Extensive serous infiltration over buttocks and between the muscles of the thighs. Aponeuroses, and muscles themselves, for the depth of two lines, and over an area of an inch and a half in diameter, intensely congested. *Blood* of both sides of the heart contained moderate number of minute rod-bacteria.

Case IX.

June 2, 1875, received from Dr. Harvey S. Gay a fine, fresh specimen of diphtheritic membrane, one-eighth of an inch thick. It was composed of fibrinous material containing few, if any, leucocytes, multitudes of minute oil-globules, but no actively moving bacteria.

No history of the case was furnished.

a. Inoculation of muscular tissue with pure membrane.

Experiment 41 :

June 2, 1875. Both thighs of a rabbit inoculated with the pure membrane.

July 1. Lump on each side as big as a filbert. That on the left side has ulcerated and is discharging yellow matter. The material of both composed of leucocytes, granular matter and a few rod-shaped and ball-headed bacteria.

July 10. Animal in excellent condition.

b. Hypodermic injection of aqueous infusion of pure membrane after filtration through porous clay.

An aqueous infusion of the membrane was made and filtered the same day through the porous clay cylinder. (See ante, page 681.) The filtrate was perfectly clear.

Experiment 42 :

June 2, 1875. Five minims of the clear filtrate injected into the right thigh, and four minims into the left, of a rabbit.

June 22. Animal somewhat thin but healthy ; fur clean ; feeding well ; no lumps to be felt.

June 29. Found dead.

June 30. *Autopsy :* Body in good condition. No sign of a lesion discoverable. (N. B.—The weather has been intensely hot.)

Experiment 43:

June 2, 1875. Six minims of the clear filtrate injected into the right thigh, and five into the left, of a rabbit.

June 22. Animal somewhat thin but healthy ; no lumps to be felt in either thigh.

July 10. Animal in good condition.

c. Hypodermic injection of unfiltered aqueous infusion of pure membrane.

The turbid infusion of "*b*," after the heavier particles had settled, was used for the injections.

Experiment 44 :

June 2, 1875. Six minims of the turbid infusion injected into each thigh of a rabbit.

June 26. Animal in good condition. On each side, a lobulated hard lump, as big as a filbert, apparently not connected with the muscular tissue. Pricked, a yellowish white curdy matter exuded, found to be composed of coarsely granular leucocytes, granular matter and rod-bacteria.

July 10. Animal in good condition.

Experiment 45 :

June 2, 1875. Six minims of the turbid infusion injected into each thigh of a rabbit.

June 5. Animal died during night. *Autopsy.—Right thigh:* General congestion of connective tissue and aponeurosis of thigh-muscles, with presence of extensive clots of blood, apparently from injury to a vessel by the hypodermic needle. At one spot, a circle of yellow infiltration of the connective tissue half an inch in diameter. Through the aponeurosis underlying this patch, a small hole could be traced, and on exposing the muscle beneath, the latter presented a ring of yellow infiltration corresponding in position with that of the patch in the connective tissue above, in the centre of which was a grayish, sloughing cavity. Apparently, the needle had forced the muscular tissue. The yellow infiltration of the tissues contained abundant leucocytes and living rod-bacteria, and the gray pultaceous muscular slough was composed of muscular tissue undergoing fatty degeneration, and swarming with living rod-bacteria. *Left thigh:* Appearance the same except that there was no lesion of the muscle. *Blood*, from both sides of the heart, contained no bacteria.

d. Hypodermic injection of aqueous infusion of pure membrane ten days old and putrid.

The unfiltered infusion of "*c*" was allowed to stand for ten days. Evaporation was compensated for by adding two fluid drachms of distilled water. The infusion was now thoroughly putrid, being a turbid brown fluid, having an extremely offensive smell, and swarming with actively moving bacteria.

Experiment 46:

June 12, 1875. Six minims of the putrid infusion injected into each thigh of a rabbit.

June 13. Found dead on the floor, having escaped from its cage and fallen a considerable height. *Autopsy:* Body limp; hind legs have an offensive smell, but not the fore-legs. *Left thigh:* No lesion except faint pinkish discoloration in connective tissue at site of injection. A bit of the lax connective tissue examined microscopically, appeared normal, except that there were numerous rod-bacteria to be seen. *Right thigh:* Same condition, bacteria far more numerous. *Liver:* Large clot on under surface; organ passively congested. *Kidneys:* Congested. *Blood* of right heart fluid and containing an exceedingly small number of minute rod-bacteria.

Experiment 47 :

June 12, 1875. Six minims of the putrid infusion injected into each thigh of a rabbit.

July 10. Animal in good condition. Skin snipped over both thighs and parts explored. *Right thigh:* Small flattened sac, about ⅜-inch in diameter, covered with newly formed vessels, found in the subcutaneous connective tissue. Sac when opened, found to contain about a drop of soft yellowish material, composed of leucocytes, flattened corpuscles stuffed with oil-globules, and an immense amount of free oil-globules aggregated together here and there into chains. No bacteria. *Left thigh:* Appearances same, but more free oil and a few motionless rod-bacteria.

e. Hypodermic injection of putrid infusion of pure membrane, after filtration through paper (single filter).

The putrid infusion of "*d*" was filtered through a single ordinary paper filter. The filtrate was clear, but of a yellow color, foul smelling, and contained a few actively-moving bacteria in each microscopic field.

Experiment 48:

June 12, 1875. Six minims of the filtrate injected into each fore leg of a rabbit.
June 24. Animal in excellent condition; no lump to be felt in either leg.
July 10. Animal in excellent condition.

Experiment 49:

June 12, 1875. Six minims injected into each thigh of a rabbit.
July 10. Animal in excellent condition. No lump to be felt in either thigh.

f. Hypodermic injection of putrid infusion after boiling.

Some of the putrid infusion of "*d*" was boiled in a test-tube for three minutes and allowed to cool. All the contained bacteria were then motionless.

Experiment 50:

June 12, 1875. Six minims of the boiled infusion injected into each thigh of a rabbit.
June 18. Found dead. *Autopsy:* Body emaciated. *Right thigh:* Small, rather thick, patch, one-third by one-eighth inch in area, in the connective tissue at site of injection. Material composed of large flattened corpuscles filled with oil-globules and granules, but no bacteria; the muscle beneath in a state of fatty degeneration. *Left thigh:* Soft, yellow patch in connective tissue at sight of injection, the size of a three-cent piece. Composition same as above.

Experiment 51:

June 12, 1875. Six minims of the boiled infusion injected into each thigh of a rabbit.
June 29. Found dead. No autopsy.

Case X.

June 5, 1875, received from Dr. W. H. Vermilye a piece of diphtheritic membrane detached by coughing on the seventh day of the disease. The patient, a girl six years old, was attacked with what at first seemed to be a simple follicular tonsillitis. On the sixth day the tonsils, palate, and pharynx became covered with a thick exudation, which peeled from the palate quite easily, leaving a bleeding surface behind. The next day the same surfaces became again covered, but thinly and in patches. Some of these patches were pulled upon and loosened, and immediately afterward the sample furnished was detached and discharged by expectoration. The patient had already begun to improve, and without having had any symptoms of laryngeal obstruction or of constitutional infection, made a good recovery after two weeks illness. Albumen was detected in the urine on the twelfth day, but the urine was not examined regularly, the patient being a dispensary case. Two sisters of the patient were also attacked, but with them the disease was of even a milder type. The specimen was

received wrapped in moist rag, encased in oiled silk. It was not opened till the second day after receipt. It was then yellowish-red, soft, and pultaceous, and had a putrid smell. Microscopically, it was found to consist of corpuscles of various forms matted together and infiltrated with dense masses of bacteria, which swarmed in the field as free rods of various sizes.

a. Hypodermic injection of aqueous infusion of pure membrane.

Eighteen grains weight of the membrane was ground up with three fluid drachms of water and infused one hour. The turbid, supernatant fluid, after settlement of the larger pieces of membrane, was used for the injections. It had no smell.

Experiment 52:

June 7, 1875. Six minims of the turbid infusion injected into each thigh of a rabbit.

June 10. Died in the previous night. *Autopsy* in the afternoon: Subcutaneous connective tissue over whole region of each thigh hyperæmic, œdematous, and diffusely yellow. Yellow spots, composed of connective-tissue densely stuffed with bacteria in closely-packed masses. Swarms of bacteria, both dumb-bell and rod forms, actively moving about in the field of the microscope. *Blood*, of right heart, contained no bacteria. *Kidneys:* Tubes contained a great deal of fat in small granules. In the free fluid were numerous bodies, in pretty active motion, resembling bacteria. *Spleen:* Normal. *Liver:* Quite fatty.

Experiment 53:

June 7, 1875. Six minims of the turbid infusion injected into each thigh of a rabbit.

June 10. Died in the afternoon. *Autopsy* immediately: Both thighs in same condition in all respects as foregoing, only the yellow matter in this case contained, in addition to the elements above described, many leucocytes in a state of degeneration. *Blood* from right heart contained no bacteria. *Liver:* Cells slightly fatty. *Kidneys:* Tubes normal; no bacteria.

Experiment 54:

June 7, 1875. Six minims of the turbid infusion injected into each thigh of a rabbit.

June 10. Died during previous night. *Autopsy* in the afternoon.—*Right thigh:* Skin at site of injection gangrenous for an area as large as a three-cent piece; great infiltration of subcutaneous connective tissue. Muscular tissue for an area two inches in diameter reddened by small punctate spots; the tissue at these spots found to consist of broken down muscular fibres, but with no bacteria present. *Left thigh:* Appearances similar, except in addition a thin, yellow patch in the subcutaneous connective tissue, found to consist of connective-tissue elements, with leucocytes, large cells containing oil-globules and vast numbers of bacteria in active motion. Bacteria were also found among the muscular fibres in the affected spots in the muscle. *Blood* from right heart contained a moderate number of bacteria. *Kidneys:* Normal, but fluid scrapings from a cut surface contained a large number of free bacteria. *Liver:* Large and fatty. *Spleen:* Normal.

b. Hypodermic injection of aqueous infusion of pure membrane after filtration through paper (double filter).

The infusion of "*a*" was filtered through a double filter of ordinary filtering-paper, made by inserting one filter within another, so that four thicknesses of paper were everywhere presented to the fluid. The filtrate was clear and odorless, but of a yellow color.

Experiment 55 :

June 7, 1875. Six minims of the clear filtrate injected into each thigh of a rabbit.

July 1. No sign of a lump to be felt on either thigh ; no adhesion of skin to parts beneath ; animal fat and healthy.

July 10. Animal in good condition.

Experiment 56 :

June 7, 1875. Six minims of the clear filtrate injected into each thigh of a rabbit.

July 1. No sign of a lump to be felt on either thigh. Animal in excellent condition.

July 10. Animal in good condition.

Experiment 57 :

June 7, 1875. Six minims of the clear filtrate injected into each thigh of a rabbit.

July 1. Animal thin, but apparently healthy. *Right thigh :* No lump to be felt. *Left thigh :* Large lump, the size of a pullet's egg. Pricked, was found to contain the usual yellow creamy matter, composed of malformed leucocytes, granular matter, with some moving bacteria.

July 10. Animal in good condition.

Case XI.

June 19, 1875, received from Dr. Alexander Buchanan a small scrap of membraniform material taken from the throat of a scarlet fever patient, on the ninth day of the disease. The patient, a boy six years old, was first seen by Dr. B. on the eighth day of the disease. The pulse was then 188, temperature 104° Fahr. ; there was enormous swelling of the parotids, and from the tonsils upwards to the posterior nares the mucous membrane was covered with exudation. The small piece furnished was removed the next day. Patient died on the fourteenth day, having from the first refused all nourishment. The specimen of membrane was about 1-16 inch thick, greenish yellow in color, and so friable as to be easily broken into pieces. Microscopically, the material was composed, in greatest part, of the thalli and spores of a fungus, apparently penicilium glaucum. Rod-bacteria and deformed, flattened corpuscles, probably lymphoid cells, were also present in considerable number. There was only enough of the material for the inoculation of two animals.

a. Inoculation of muscular tissue with membrane from scarlet fever patient.

Experiment 58:

June 20, 1875. Both thighs of a rabbit inoculated with the solid membrane.

July 4. Dying. *Right thigh* showed a small lump, containing cheesy material, as in cases where pure diphtheritic membrane was inoculated.

Experiment 59:

June 20, 1875. Both thighs of a rabbit inoculated with the solid membrane.

July 10. Animal in good condition, but somewhat thin. *Left thigh:* Small lobulated yellow tumor in the connective tissue, at the site of injection. Mass composed of deformed leucocytes, large, flat corpuscles, containing fat and granular débris. No rod-bacteria. *Right thigh:* No lump to be felt.

Case XII.

June 19, 1875, received from Dr. A. S. Church a second piece of diphtheritic membrane. The patient, a boy sixteen months old, was taken with fever and convulsions. Dr. C. saw him first on the third day, and membrane was then already present upon the tonsils and uvula. It subsequently appeared in the nose. Small pieces were detached on the sixth day, and larger ones on the ninth. There was no obstruction of the larynx, but there were constitutional symptoms, with albumen, and granular and blood-casts in the urine. Recovered, after three weeks' illness. The specimen furnished was a broad, leathery strip, over two inches long, nearly an inch wide, and from 1-16 to 1-8 inch thick. It was made up of a distinctly fibrillated network, with flattened corpuscles, containing oil-drops. Numerous bacteria were also present.

a. Inoculation of muscular tissue with pure membrane.

Experiment 60:

June 20, 1875. Both thighs of a rabbit inoculated with pure membrane.

June 26. Animal thin, but healthy. No lumps to be felt at sites of inoculation.

July 10. Animal in good condition.

Experiment 61:

June 20, 1875. Both thighs of a rabbit inoculated with pure membrane.

June 26. Animal thin, but healthy. No lumps to be felt.

July 8. Killed by a fall from a considerable height.

July 9. *Autopsy.—Left thigh:* Small lump. *Right thigh:* Nothing to be found.

Case XIII.

January 21, 1876, received from Dr. Francis H. Weismann a piece of diphtheritic membrane, coughed up by a patient on the sixth day of the disease. The patient, a boy eleven

years old, was taken with slight chills and cough, followed by swelling of the glands and high fever. On the fourth day membrane formed in the throat, involving the cavity of the mouth and extending into the larynx, producing obstruction to breathing. Pieces were first coughed up on the sixth day, and from time to time many large pieces were thus detached. The prognosis was bad from the first, the urine being slightly albuminous and constitutional symptoms present. A small cut on the finger became covered with membrane. Paralysis was already beginning in the right fore-arm when the patient died, on the fourteenth day of the disease. Others of the same family were beginning to show symptoms of diphtheria when this boy died. The specimen of membrane was examined two days after detachment. It was then firm, tenacious, of a yellowish-white color, and was made up principally of the interlacing filaments of the mycelium of a fungus. There were also numerous minute spherical bodies, but no rod-bacteria.

a. Inoculation of muscular tissue with pure membrane.

Experiment 62 :

January 22, 1876. Both thighs of a rabbit inoculated with the pure membrane.

January 25. Found dead in the morning. Animal had partly succeeded in getting out of his cage.

January 26. *Autopsy* in the afternoon.—*Right thigh :* Spreading from site of inoculation, a diffuse inflammation, the subcutaneous connective-tissue being infiltrated with yellow pasty material, and the veins of the region congested. The appearances, in short, were the same as have been so often noted, only the lesions were not severe. The yellow material was composed of swollen, distorted corpuscles, with granular débris. *Left thigh :* Diffuse inflammation of connective-tissue, and fatty degeneration of the muscle at site of inoculation. The strings of fat granules between the muscular fibres looked much like round bacteria, but many were too large, and others were dissolved by even a weak solution of caustic potash. *Viscera* normal.

Experiment 63 :

January 22, 1876. Both thighs of a rabbit inoculated with pure membrane.

January 24. Found dead in the morning. *Autopsy :* Microscopic appearances same as in foregoing case. No microscopic examination made

b. Inoculation of muscular tissue with mixture of pure membrane and salicylic acid.

Fourteen grains weight of the membrane was triturated with fourteen drops of water, containing in solution two per cent. of salicylic acid and six per cent. of sodic phosphate. Next, five grains weight of salicylic acid itself was added, and the whole mixture thoroughly triturated in a mortar for half an hour.

Experiment 64 :

January 22, 1876. Both thighs of a rabbit inoculated with the mixture of membrane and salicylic acid.

January 26. Animal in good condition.

January 29. Animal in good condition.

February 22. Killed in a fight with another rabbit. There were lumps found at the site of inoculation.

Experiment 65 :

January 22, 1876. Both thighs of a rabbit inoculated with the mixture of membrane and salicylic acid.

January 29. Animal in good condition.

February 22. Animal in good condition.

February 25. Animal in good condition.

March 11. Animal in very good condition. Very small lumps to be felt on both sides at sites of inoculation.

Experiment 66 :

January 22, 1876. Both thighs of a rabbit inoculated with the mixture of membrane and salicylic acid.

January 26. Animal in good condition.

February 23. Animal in good condition. Small lumps to be felt on both sides at sites of inoculation.

March 11. Animal in fair condition, though rather thin. Lumps still to be felt.

Case XIV.

February 25, 1876, received from Dr. William E. Bullard two pieces of diphtheritic membrane. The patient, a boy six years old, had had enlarged tonsils for some weeks. Next, the whole pharyngeal surface became very red, and the following day membrane formed upon both tonsils, uvula, and anterior nares. Four days later much of the membrane came away, leaving the left nostril and one of the tonsils free. At this date the temperature was high, the pulse irregular, and the prostration great. But, in six days more, all signs of membrane had disappeared, and the patient recovered after an illness, in all, of thirteen days. A sister of this boy was in the fourth day of an attack of diphtheria when he himself first fell ill. The larger specimen of membrane was about one-eighth inch thick, soft, friable, and of a grayish-yellow color. It consisted of a fibrillated matrix containing large numbers of corpuscles of various sizes, and also numerous bacteria. The smaller specimen was still more distinctly fibrillated; contained large numbers of granules, but few bacteria.

a. Inoculation of muscular tissue with pure membrane.

Experiment 67 :

February 25, 1876. Both thighs of a rabbit inoculated with pure membrane.

February 28. Animal in good condition.

March 11. Ulcers at sites of inoculation, discharging freely. Animal fat, and seemingly in good condition.

Experiment 68 :

February 25, 1876. Both thighs of a rabbit inoculated with the pure membrane.

February 28. Animal in good condition.

March 11. Animal very weak but has good appetite. Open ulcers at sites of inoculation, discharging freely an offensive matter.

March 19. Dead. *Autopsy:* Body greatly emaciated. Abscesses over both thighs, extending into the muscular tissue; contents consisting of pus-corpuscles, large granular corpuscles and granular matter, but no bacteria.

Experiment 69:

February 25, 1876. Both thighs inoculated with pure membrane.

February 28. Animal in good condition.

March 11. Animal thin, but pretty active. Coat rather dirty and rough. Ulcers at sites of inoculation discharging freely an offensive matter.

June 13. Animal in good condition. Ulcers healed; no lumps or thickening to be felt.

b. Inoculation of muscular tissue with mixture of pure membrane and salicylic acid.

Seven grains weight of membrane was treated first with salicylic acid solution, and then with the solid acid in exactly the same manner and proportion described in the last case. (See ante, page 691.)

Experiment 70:

February 25, 1876. Both thighs of a rabbit inoculated with the mixture of membrane and salicylic acid.

February 28. Animal in good condition.

February 29. Found dead at 3 P. M.

March 2. *Autopsy:* Moderate sized collections of yellow material at sites of inoculation in the muscles, but not much inflammation of surrounding tissues.

Experiment 71:

February 25, 1876. Both thighs of a rabbit inoculated with the mixture of membrane and salicylic acid.

February 28. In good condition.

March 11. Found dead. *Autopsy.—Left thigh:* Large patch at site of inoculation; matter pasty in consistence and of a rather reddish tinge. Considerable inflammation of surrounding tissues. Internal organs apparently healthy. Body much emaciated.

Experiment 72:

February 25, 1875. Both thighs of a rabbit inoculated with the mixture of membrane and salicylic acid.

February 28. Animal in good condition.

March 11. Large lumps on both sides at sites of inoculation. Had begun to ulcerate and discharge externally. Contents white, creamy and almost odorless.

II.—INOCULATION OF SCRAPINGS FROM THE HEALTHY HUMAN TONGUE.

a. Inoculation of muscular tissue with pure tongue-scrapings.

The upper surface of a healthy human tongue with an habitual tendency to be "furred," was thoroughly scraped with a knife. The pulpy yellowish-white scrapings consisted of the ends of the filiform papillæ covered by epithelium, with the latter in turn densely covered and infiltrated with packed layers of bacteria. The scrapings were used at once for inoculation.

Experiment 73 :

April 29, 1875. One thigh of a rabbit inoculated with fresh tongue-scrapings.

May 3. Dead. *Autopsy :* Large lump of dirty yellow color at site of inoculation ; material containing free bacteria and also round balls or nests of cloudy packed bacteria exactly similar to those seen in diphtheritic membrane. *Blood* contained only a few bacteria.

Experiment 74 :

April 29, 1875. Both thighs of a rabbit inoculated with fresh tongue-scrapings.

May 4. No effect observed.

May 7. Lump felt at site of inoculation on one side. Skin snipped and parts explored. A flattened yellow tumor, the size and shape of a kidney-bean, found in the connective-tissue, immediately over the muscle-wound. By pricking its thickened wall of connective-tissue, a white pasty substance was obtained, found to consist of broken down leucocytes, some rod-bacteria and an immense amount of granular matter. No balls of bacteria were to be seen. Tumor left in situ.

May 17. Very considerable general swelling of thigh and leg on the left side, and fluctuation detected over this whole area as far, even, as the toes. Animal evidently sickly.

May 24. Dead. *Autopsy.—Right thigh :* An extensive cavity, whose contents had been discharged by ulceration, found under the skin, extending over the whole area of the thigh. The skin itself formed the outer wall of the sac, and internally the muscles and periosteum had disintegrated so as to expose nearly the whole length of the femur, and part of the ilium, denuded, at the bottom of the sac. *Left thigh :* A large livid, flattened, fluctuating tumor, covering the whole area of the thigh and leg, and reaching to the toes. Tumor consisted of a wall of thickened connective-tissue enclosing a dirty, yellowish, semi-fluid material, consisting of deformed leucocytes, granular matter, and large numbers of actively moving bacteria. Examination of *abdomen* showed general peritonitis, loops of intestine being adherent to the abdominal wall and to each other by fresh, moderately firm, adhesions. *Liver :* Deeply congested, and containing echinococcus ova. *Lungs :* Deeply congested. *Blood :* From right heart, vena cava, and from lungs, liver, and kidney contained a moderate number of rod-bacteria of larger size than common, but none of these elements were found in blood taken from the left heart.

Experiment 75:

April 29, 1875. Both thighs of a rabbit inoculated with fresh tongue-scrapings.

May 4. Animal ing ood condition; small lumps to be felt at each site of inoculation. Pricked, and contents found to be a yellow pasty material, composed of broken down leucocytes, granular matter, actively moving rod-bacteria, and balls of closely-packed bacteria.

May 7. Appearances same in all respects, but in addition there are open ulcers at the sites of the skin-wounds.

May 12. Dead. Body considerably emaciated. The lumps have increased a good deal in size.

b. Inoculation of muscular tissue with pure tongue-scrapings.

Tongue-scrapings obtained in the same way as in "*a*," but twenty-four hours after removal, were used for the following inoculations. The material had already acquired an offensive smell.

Experiment 76:

May 6, 1875. Both thighs of a rabbit inoculated with pure tongue-scrapings.

May 7. Skin over right thigh snipped and parts explored. Oblong yellow tumor, one inch long by three-eighths of an inch wide, found connecting the skin and muscle-wounds. On opening the tumor, the contents were found to be a yellow pasty material, made up of leucocytes and enormous multitudes of bacteria. Débris of the inoculated material could also be found in the mass. The entire tumor, walls and all, was carefully dissected out and bodily removed.

June 24. Animal in good condition. No lumps to be felt in either thigh.

July 10. Animal in good condition.

Experiment 77:

May 6, 1875. Both thighs of a rabbit inoculated with pure tongue-scrapings.

May 9. Skin of right thigh snipped and parts explored. Skin-wound closed; yellow oblong tumor connecting skin and muscle-wounds; contents white and semi-fluid containing myriads of bacteria. A drop of blood, obtained by pricking a vein in the ear, showed no bacteria.

May 25. Dead. *Autopsy.—Right thigh:* The above-mentioned tumor found to measure one inch by one-eighth inch in area; material consisted of leucocytes undergoing fatty degeneration, and a great deal of free fat in globules, but no bacteria were now to be seen. *Left thigh:* The whole muscular tissue was infiltrated with distinct collections of yellow, semi-fluid material, consisting of leucocytes undergoing fatty degeneration, and a moderate number of rod-bacteria, the latter being most numerous in the foci nearest the skin-wound. *Blood* contained no bacteria.

Experiment 78:

May 6, 1875. Both thighs of a rabbit inoculated with pure tongue-scrapings.

May 10. Skin of left side snipped and parts explored. Yellowish-white tumor an inch long connecting skin and muscle-wounds; contents, leucocytes undergoing fatty degeneration and a moderate number of free bacteria. No "balls" of bacteria.

May 19 Collections of yellowish matter in both thighs which have ulcerated externally and have been discharging. Site of ulcers now covered by scabs. Discharged matter consisted of modcrate number of leucocytes and abundant and active bacteria.

May 31. Dead.

June 1. *Autopsy.—Left thigh:* Multilocular tumor in the subcutaneous connective-tissue, as large as a large filbert; not attached to the aponeurosis of the muscle, nor could the muscle wound be found. Contents of tumor white and semi-fluid, consisting of leucocytes undergoing degeneration, granular débris, and shreds of disorganized connective-tissue containing large numbers of minute bacteria. *Right thigh:* Presented nothing abnormal except a slight thickening of the connective-tissue immediately underlying the skin at the site of the original wound. The *visceral* cavities and organs were healthy. *Blood:* From both sides of the heart showed crenation of the red corpuscles, and a massing of the white into groups of from six to thirty. No more bacteria observable than is usual in the blood of animals thirty hours dead.

Experiment 79:

May 6, 1875. Both thighs of a rabbit inoculated with pure tongue-scrapings.

May 10. Animal sick.

May 11. Dead. *Autopsy* between 12 and 17 hours after death.—*Left thigh:* Large flat collection of white pasty material in the subcutaneous connective-tissue, an inch in diameter; material composed of granular leucocytes, free granules and rod-bacteria in considerable number. At site of muscle-wound the muscular tissue showed close lines of white glistening aspect running parallel to the direction of the muscular fibres. These consisted of the sarcolemma of muscular fibres filled with closely packed granular débris in place of the usual sarcous substance. Brightly shining spherical bodies (too glistening for bacteria) of varying size, occasionally arranged in rows, also appeared scattered in the sarcous substance of the muscular fibres near these streaks of white. *Right thigh:* Large diffuse collection of yellowish-white material of irregular shape, measuring three by one-and-a-half inches in area, in the subcutaneous connective-tissue. This patch was attached to the skin and the muscle at the sites of the original wounds in the same. Material composed of connective-tissue elements infiltrated with degenerating leucocytes and enormous numbers of bacteria. The cavity of the muscle-wound contained a dirty pasty substance in small quantity, consisting of broken down muscular débris and closely packed masses of bacteria. Around this spot were white streaks similar to those above described as found in the other thigh. *Blood:* From a vein in the neighborhood of the muscle-wound showed no bacteria, but that from the right heart contained bacteria. There were general *pericarditis*, *peritonitis*, and inflammation of the tunica vaginalis of the *testicles* with fibrinous friable soft exudation, composed of a fibrinous network in whose meshes were myriads of bacteria.

c. Inoculation of muscular tissue with mixture of tongue-scrapings and salicylic acid.

Six grains' weight of pulpy tongue-scrapings, three hours after removal, was thoroughly triturated with one grain of Squibb's unbleached salicylic acid.

Experiment 80:

May 28, 1875. Both thighs of a rabbit inoculated with the mixture of tongue-scrapings and salicylic acid.

June 4. Animal extremely emaciated and dull. Thick lump on each side at sites of inoculation. Pricked, exuded yellow semi-fluid matter consisting of granular leucocytes, granular débris, and a few *motionless* rod-bacteria.

June 5. Dead. By a mistake the body was thrown away before an *autopsy* was made.

Experiment 81:

May 28, 1875. Both thighs of a rabbit inoculated with the mixture of tongue-scrapings and salicylic acid.

June 4. Animal in fine condition. Skin of right thigh snipped and parts explored. Large thick lump an inch long and half an inch wide. Contents pulpy, consisting of very granular leucocytes, but no bacteria. On left side a smaller thickening could be felt under the scab of the skin-wound. Pricked, exuded pulpy yellow matter, consisting of granular leucocytes, and a very small number of *motionless* bacteria.

June 13. Dead. *Autopsy* four hours after death: Animal had been in good condition though somewhat thin; fur in good order. *Left thigh:* Yellow tumor over two inches long and half an inch thick and wide, connecting the skin and muscle-wounds; thickest over muscle-wound; composition same as found June 4; no bacteria. No signs of circumjacent serous infiltration or congestion. *Right thigh:* Exactly the same condition of things; the tumor was nearly two inches long, and half an inch wide and thick. *Blood* of right ventricle contained a very few club-shaped bacteria, motionless. The red globules in many instances were beset with processes projecting radially and having exactly the appearance of short rod-bacteria. But on adding water these processes became pale like the rest of the substance of the corpuscle, and as the latter swelled under the action of the water these processes were rapidly retracted, till they disappeared wholly, leaving the usual smooth, round contour to the swollen corpuscle.

Experiment 82:

May 28, 1875. Both thighs of a rabbit inoculated with the mixture of tongue-scrapings and salicylic acid.

June 4. Animal in fine condition. Thick lump felt on each side. Pricked, exuded yellow semi-fluid matter containing leucocytes and granular débris, but no bacteria.

June 22. Animal in fine general condition. On the right side the lump is as large as a pigeon's egg. On squeezing, a yellowish material exuded from under the scab covering the skin-wound. Contained granular leucocytes and débris, but no bacteria.

July 10. Animal in excellent condition.

d. Inoculation of muscular tissue with putrefying tongue-scrapings.

The scrapings of five successive mornings were mixed as obtained, and used for inoculation on the fifth day. The mixed material had acquired a horribly offensive smell.

Experiment 83:

January 4, 1876.. Both thighs of a rabbit inoculated with the fœtid tongue-scrapings.

January 10. Lumps felt on both sides.

January 13. The lumps have ulcerated externally; discharging freely an extremely foul, offensive matter. Animal not as lively as others, but fat, and fur in good condition.

January 26. Animal in good condition.

February 22. Killed in a fight with another rabbit. *Autopsy:* Large collection of the usual matter at sites of inoculation, but no lesion of any internal organs.

Experiment 84:

January 4, 1876. Both thighs of a small rabbit inoculated with the fœtid tongue-scrapings.

January 10. Dead. *Autopsy:* On both sides the usual lumps of yellow material. (N. B.—This rabbit was one of a litter of six small animals under three months of age, two of which, not experimented upon at all, died about the same time from unknown causes.)

e. Inoculation of muscular tissue with mixtures of putrefying tongue-scrapings and salicylic acid.

Fourteen grains' weight of the fœtid tongue-scrapings of "*d*" was treated with salicylic acid solution, and then with the solid acid in exactly the same manner as the diphtheritic matter in series "*b*," Case XIII. (see ante, page 691). After mixture with the salicylic acid the excessively offensive odor of the stale tongue-scrapings almost wholly disappeared.

Experiment 85:

January 4, 1876. Both fore-shoulders of a rabbit inoculated with the mixture of tongue-scrapings and salicylic acid.

January 10. Animal in good condition. Lumps the size of a large marble on each side.

January 13. Lumps as large as a filbert on each side. Skin-wound scabbed over. On detaching the scab the contents of the lump were exposed and were found to consist of the usual yellowish pasty material. Animal in good health.

January 16. Animal in good condition.

March 11. Animal in good condition and fat. Ulcers have healed.

April 18. Animal perfectly well.

Experiment 86 :

January 4, 1876. Both fore-shoulders of a rabbit inoculated with the mixture of tongue-scrapings and salicylic acid.

January 10. Lumps on both sides the size of a large marble. Animal in good condition.

January 13. Animal in good condition. Lumps the size of a filbert, and discharging matter externally.

January 26. Animal in good health, but paralyzed.

March 11. Animal perfectly well. Ulcers healing.

Experiment 87 :

January 4, 1876. Both fore-shoulders of a rabbit inoculated with the mixture of tongue-scrapings and salicylic acid.

January 10. Condition and lumps same as the two foregoing.

January 13. Lumps the size of a small marble ; yellowish pasty matter exuding from under scab over skin-wound.

January 26. Animal in good condition.

February 23. Animal in good condition.

March 11. No marks of ulceration ; animal fat and in good health.

Experiment 88 :

January 4, 1876. Both thighs inoculated with the mixture of tongue-scrapings and salicylic acid.

January 10. Animal lively and in good condition ; small lumps on both sides.

January 13. Same condition as the others.

January 26. Animal in good condition.

February 20. Killed in a fight with another rabbit.

Experiment 89 :

January 4, 1876. Both thighs inoculated with the mixture of tongue-scrapings and salicylic acid.

January 10. Small lumps on each thigh.

January 13. Small lumps on both sides. Skin-wounds scabbed ; yellowish material easily squeezed out from under scabs.

January 23. Dead. No autopsy.

Experiment 90 :

January 4, 1876. Both thighs of a rabbit inoculated with the mixture of tongue-scrapings and salicylic acid.

January 10. Small lumps, but with boundaries not very well defined, on both sides.

January 13. Large scabs at site of skin-wounds ; yellow creamy matter exuding from under the scabs of nearly as offensive odor as in Experiment 83.

January 16. Dead. No autopsy.

III.—INOCULATIONS OF DECOMPOSED COHN'S SOLUTION.

a. Inoculation of muscular tissue with sediment from bottle of decomposed Cohn's solution.

A large bottle of Cohn's solution[1] was prepared and allowed to spontaneously decompose. It became turbid, acquired a strong putrid smell, and deposited a thick pulpy sediment. Fluid and sediment swarmed with actively moving bacteria. The sediment was separated by straining and used for the inoculations.

Experiment 91 :

May 15, 1875. Both thighs of a rabbit inoculated with the pulpy sediment from the decomposed Cohn's solution.

May 19. Skin of right thigh snipped and parts explored. Thick yellowish tumor, one-fourth inch wide, connecting skin and muscle-wounds ; material composed of granular leucocytes and a moderate number of free rod-bacteria.

May 27. Right leg again explored. The yellow tumor has considerably increased in size; leucocytes undergoing fatty degeneration ; bacteria very few. Left leg explored : long yellow mass of the usual character, over 1 inch long by ¼ inch wide ; contents contain granular leucocytes and a very moderate number of bacteria.

June 8. The patches still to be felt from without. Skin not opened.

June 22. Left leg explored : the tumor has shrunk to a thin yellowish streak running from the site of the muscle-wounds forwards into the connective-tissue elements infiltrated with minute oil-globules. Right leg explored : Thin yellowish layer attached to inner surface of skin-wound. Site of muscle-wound not discoverable. Animal thin but otherwise seemingly healthy.

July 8. Died suddenly. *Autopsy.—Right thigh :* Small, very thin yellow deposit just under the skin. *Left thigh :* No action to be found. (N. B.—The weather at this time was intensely hot, and animals that were not experimented upon died.)

Experiment 92 :

May 15, 1875. Both thighs of a rabbit inoculated with the pulpy sediment from the decomposed Cohn's solution.

June 8. Animal in excellent condition ; no lumps felt. *Right leg :* Explored in the usual way ; nothing near the skin-wound, but at the muscle-wound was a lobulated yellow tumor, the size of a medium-sized pea, lying in the subcutaneous connective-tissue, and connected through the original cut in the aponeurosis with the muscular substance below. Material of the tumor contained leucocytes, minute oil globules, and large sacs stuffed with the same. Only here and there an occasional motionless rod-bacterium. No inflammation or congestion about the tumor. *Left leg* explored : Flat yellow patch in the connective-tissue near skin-wound ; material elastic and coherent ; contains connective-tissue elements undergoing fatty degeneration, leucocytes and sacs filled with oil-globules. Only rarely a bacterium to be seen motionless. The site of the muscle-wound could not be found.

July 10. Animal in excellent condition.

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[1] Formula for Cohn's solution. Ammonic tartrate 1 gramme, potassic phosphate, magnesic sulphate, aa. 5 grammes ; calcic phosphate .05 gramme, distilled water 100 c. c.

b. Hypodermic injection of lower stratum of decomposed Cohn's solution.

The supernatant fluid of a quart bottle of decomposed Cohn's solution that had stood for two months was carefully drawn off and the thick semi-fluid lowest stratum used for the injections. From four to ten minims was the quantity injected in each limb.

Experiment 93:

March 6, 1876. Both thighs injected with decomposed Cohn's solution.

April 13. Lumps on each side as big as pullet's eggs.

Experiment 94:

March 6, 1876. Both thighs injected with decomposed Cohn's solution.

March 26. Dead.

March 28. *Autopsy* 48 hours after death: Lumps on both sides; material contained leucocytes and bacteria in tolerable abundance.

Experiment 95:

March 6, 1876. Both thighs injected with decomposed Cohn's solution.

April 13. Lumps on both sides; material contained leucocytes, bacteria and granular matter.

Experiment 96:

March 6, 1876. Both thighs injected with decomposed Cohn's solution.

March 26. Lumps on both sides.

April 13. Lumps have disappeared. Animal well.

c. Hypodermic injection of decomposed Cohn's solution.

A sample of Cohn's solution was inoculated, upon making, with a few drops of old, decomposed Cohn's solution, and allowed to stand one month. It was then itself decomposed, had a putrid smell, was turbid, and swarmed with bacteria. The supernatant fluid was used for the injections, sixteen minims being injected into each limb.

Experiment 97:

April 18, 1876. Both thighs of a rabbit injected with the decomposed Cohn's solution.

April 25. Animal in fair condition; somewhat thin, and coat not so sleek as others. Perhaps small lumps, but not very distinct.

May 2. Animal in good condition. Right side lump size of pea. Left side lump size of bean. Latter pricked, exuded yellow material, containing granular and fatty leucocytes, and débris; no plainly marked bacteria.

May 9. No change.

May 22. Both lumps seemingly disappeared. Left leg explored, and yellowish thickened patch of connective-tissue found at site of wound.

June 13. Nothing to be felt.

Experiment 98:

April 18, 1876. Both thighs of a rabbit injected with the decomposed Cohn's solution.

April 25. Animal in excellent health. Apparently small lumps on both sides.

May 2. Lump on each side size of bean. Left leg explored: Material yellowish, pasty composition, as in foregoing, but more shreds of connective-tissue. Animal in good condition.

May 9. Right lump disappeared. Left, now only a small diffuse thickening.

May 22. All thickening gone.

June 13. Nothing to be felt.

Experiment 99:

April 18, 1876. Both thighs of a rabbit injected with the decomposed Cohn's solution.

April 25. Animal hearty, apparently. Small lumps in both thighs.

May 2. Animal in good condition. Right side, no lump to be felt; left side, diffuse lumpy thickening of the size and shape of a Lima bean; composition as in Experiment 97.

May 9. Lump on left side same size.

May 22. Lump on left side slightly smaller.

June 13. Lump on left side diminished to size of split pea.

Experiment 100:

April 18, 1876. Both thighs of a rabbit injected with the decomposed Cohn's solution.

April 26. Animal thin, but otherwise well. Lumps on both sides.

May 2. Left side, lump twice the size of a bean, with ulcerated and scabbed skin over area ¼ inch long by ⅛ inch wide. On lifting top of scab pasty material exuded, containing granular and fatty leucocytes and débris and swollen minute oblong bodies, possibly bacteria. Right side, lump the size of a marble, not opened. Animal thin and mangy.

May 9. Right side, lump size of bean. Left side, slight thickening only.

May 22. Right side, lump size of small pea. Left side, thickening disappeared.

June 13. Right lump also disappeared.

d. Hypodermic injection of decomposed Cohn's solution after filtration through porous clay.

Some of the same sample of Cohn's solution as was used in "*c*" was filtered through the porous clay cylinder (see ante, page 701). Two minims of fluid was obtained. This filtrate was clear, odorless, and wholly free from bacteria or other solid elements.

Experiment 101:

April 18, 1876. The two minims of clear filtrate was injected into the right side of a rabbit.

April 26. Animal in excellent condition. No sign of a lump.

May 2. Animal in fine condition. Nothing to be felt.

June 13. Same as on May 2.

e. Hypodermic injection of decomposed Cohn's solution, after filtration through many thicknesses of paper.

The same sample of Cohn's solution used in "*c*" and "*d*" was filtered through successive paper filters as follows, a fresh filter or filters being taken each time: Single, twice; double, twice; triple, once; quadruple, four times. The filtrate was faintly opalescent only, and had entirely lost the original putrid smell. It contained but a few active bacteria.

Experiment 102:

April 18, 1876. Eight minims of the filtrate injected into each thigh of a rabbit.

April 25. Animal in excellent condition. No lumps to be felt.

May 2. Same as on April 25. Left leg explored; nothing to be found.

June 13. Everything perfectly normal.

Experiment 103:

April 18, 1876. Same procedure; same history; same results as in Experiment 102.

Experiment 104:

April 18, 1876. Same procedure; same history; same results as in Experiment 102.

Experiment 105:

April 18, 1876. Same procedure; same history; same results as in Experiment 102.

f. Hypodermic injection of decomposed Cohn's solution.

A sample of Cohn's solution was inoculated with one fluid ounce of old decomposed Cohn's solution. It was used for the following injections three weeks later, was then thoroughly decomposed, smelling strongly putrid and swarming with living bacteria. Each injection was sixteen minims taken from the upper stratum of the bottle.

Experiment 106:

June 13, 1876. Both thighs injected with the decomposed Cohn's solution.

June 20. Large flat area of thickening on right side about one inch in diameter. Left side, lump the size of a bean.

June 27. The thickened patch on the right side has ulcerated; left side, no change. Animal thin and poorly.

Experiment 107:

June 13, 1876. Both thighs injected with the decomposed Cohn's solution.

June 20. Nothing to be felt on either side.

June 27. Nothing to be felt on either side.

Experiment 108:

June 13, 1876. Both thighs injected with the decomposed Cohn's solution.

June 20. Right side, very small thickening to be felt, like a string with a knot at the end. Left side, lump the size of a split pea.

June 27. Right side, thickening disappeared. Left side, lump size of a small bean.

Experiment 109:

June 30, 1876. Both thighs injected with the decomposed Cohn's solution.

June 20. Right side, considerable diffuse thickening to be felt, like a bean wrapped in cotton; left side, very little, if any thickening.

June 27. Thickening on right side disappeared; cord of thickening, like a string, developed on left side. None of the animals of this series showed any impairment of their general health.

g. Hypodermic injection of decomposed Cohn's solution after thorough aeration.

Into the nozzle of a funnel a rubber cap, pierced with a small hole, was firmly wedged. Fluid poured in the funnel thus armed escaped through the hole in the rubber plug only drop by drop, and about as fast as in ordinary filtration. Some of the same Cohn's solution used in "*f*" was then passed through this plugged funnel nine successive times. After this aeration, the solution was of course as turbid as ever, but it had entirely lost the putrid smell it originally had, a slightly mawkish odor alone remaining. Sixteen minims of this aerated solution was used in each of the following injections:

Experiment 110:

June 13, 1876. Both thighs of a rabbit injected with the aerated Cohn's solution.

June 20. Right side, nothing to be felt; left side, lump the size of a large pea.

June 27. Right side, lump size of a shot; left side, lump size of a split pea.

Experiment 111:

June 13, 1876. Both thighs of a rabbit injected with the aerated Cohn's solution.

June 20. Right side, lump size of a marble; left side, lump size of a bean.

June 27. Right side, lobular diffuse lump, size of a Lima bean; left side, slight cord-like thickening.

Experiment 112:

June 13, 1876. Both thighs of a rabbit injected with the aerated Cohn's solution.

June 20. Both sides, lumps size of a large pea.

June 27. Both sides, lumps size of a large bean.

Experiment 113:

June 13, 1876. Both thighs of a rabbit injected with the aerated Cohn's solution.

June 20. Right side, lump size of a large pea; left side, lump size of a large bean.

June 27. Both sides, lumps size of a large bean. These were pricked, and the usual yellowish creamy material exuded on pressure. None of the animals of this series suffered any impairment of their general health.

IV.—INOCULATIONS OF PUTRID INFUSION OF CALF'S LIVER.

a. Hypodermic injection of the putrid liver infusion.

Pieces of calf's liver were infused in water, and after a few days the infusion was used for experimentation. The fluid had an intensely strong putrid smell, and swarmed with living bacteria.

Experiment 114:

November 12, 1875. One thigh of a rabbit injected with the putrid infusion.

November 15. Small yellowish-white tumor found in the connective-tissue at site of injection, identical in appearance with those occurring after inoculation with diphtheritic membrane, tongue-scrapings, etc.

b. Hypodermic injection of putrid liver-infusion after filtration through porous clay.

Some of the infusion of "*a*" was drawn through the porous clay-cylinder.

Experiment 115:

November 12, 1875. One thigh of a rabbit injected with the clear filtrate (three minims).

November 15. No discoverable effect.

c. Hypodermic injection of putrid liver-infusion after filtration through paper.

Some of the infusion of "*a*" was filtered through a single paper filter, and some through a double filter, so managed that four thicknesses of paper were everywhere presented to the fluid. The filtrate in both cases showed no rod-bacteria, but multitudes of minute granules.

Experiment 116:

November 12, 1875. One thigh of a rabbit injected with the filtrate from the single filter.

November 15. No discoverable effect.

Experiment 117:

November 12, 1875. One thigh of a rabbit injected with the filtrate from the double filter.

November 15. No discoverable effect.

d. Inoculation of solid residue from putrid liver-infusion, after evaporation by boiling, and treatment with boiling absolute alcohol.

About nine fluid ounces of the unfiltered infusion was boiled continuously until it was nearly evaporated to dryness. The solid residue was then further boiled with absolute alcohol,

and the alcohol then removed by filtration. The moist residue on the filter was used for inoculation in one case, and, mixed with water, for hypodermic injection, in another case.

Experiment 118:

November 12, 1875. One thigh of a rabbit inoculated with the moist residue.

November 15. Large lump at site of inoculation, of the usual character.

Experiment 119:

November 12, 1875. One thigh of a rabbit injected with the moist residue mixed with water.

November 15. Large lump, as in foregoing.

e. Hypodermic injection of alcoholic solution from residue of evaporated putrid liver-infusion.

The alcoholic filtrate of "*d*" was used for the injection.

Experiment 120:

November 12, 1875. One thigh of a rabbit injected with the alcoholic filtrate.

November 15. No discoverable effect.

V.—INOCULATIONS OF SAND.

Sand was roasted, and allowed to cool. Some was used for inoculation, dry; some, after mixing into a pulp with a little of Liebig's extract of meat.

Experiment 121:

May 15, 1875. Both thighs of a rabbit inoculated with dry sand.

May 19. Animal in good condition; nothing to be felt. Legs explored; sand found encapsuled, each grain by itself. No yellow tumor; no signs of inflammation.

May 27. Legs explored; thin line of slightly thickened connective-tissue, studded with sand particles connecting skin and muscle-wound. No yellow tumor; no leucocytes; no bacteria.

May 31. Animal perfectly well.

Experiment 122:

May 15, 1875. Same procedure, history and results as in Experiment 121.

Experiment 123:

May 15, 1875. Both thighs of a rabbit inoculated with the mixture of sand and meat extract. Subsequent history and results same as in Experiment 121.

Experiment 124:

May 15, 1875. Same procedure, history and results as in Experiment 123.

VI.—INOCULATIONS OF SALICYLIC ACID.

a. Inoculation of muscular tissue with solid salicylic acid.

Some of Squibb's unbleached salicylic acid was mixed into a paste with water, and inoculated in the usual way.

Experiment 125:

May 28, 1875. Both thighs of a rabbit inoculated with the paste of salicylic acid.

June 4. Animal in excellent condition. Skin-wounds closed by scabs, without thickening. *Left side:* A depression felt over site of muscle-wound. Skin snipped, and parts explored. No sign whatever of any lesion in the connective-tissue. The hole made by the original muscle-wound remained as an excavation ¼ inch in diameter, presenting a brown-colored surface. The material of this brown layer of tissue consisted of connective-tissue elements, blood-corpuscles, and muscular fibres in a state of unnatural stiffness, shrinkage, and brownish discoloration—an appearance similar to what is produced in muscle by the action of weak chromic acid. No sign of pus, or of irritation of tissues in the neighborhood of the muscle-wound.

July 1. Skin and muscle-wounds completely healed. Animal in good condition.

July 10. Animal in good condition.

Experiment 126:

May 28, 1875. Both thighs of a rabbit inoculated with the paste of salicylic acid.

June 4. Skin of thighs snipped and parts explored. Appearances identical with those of foregoing experiment at same date.

June 24. Animal well; nothing to be felt from without; legs not explored.

July 10. Animal in good condition.

Experiment 127:

May 28, 1875. Both thighs of a rabbit inoculated with the paste of salicylic acid.

June 4. Animal in excellent condition. No thickening to be felt, and on opening skin, no lesion in the neighborhood of the skin-wound.

June 12. Animal in excellent health.

b. Hypodermic injection of salicylic acid dissolved in aqueous solution of sodic phosphate.

The rabbits used in this and the following series of experiments were all of the same litter; small animals, weighing 1¼ pounds each.

Experiment 128:

March 12, 1875. Injected following solution: Salicylic acid, 5 grains; sodic sulphate, 15 grains; water, 25 minims. For the following two hours animal somewhat stupid and quiet; respirations quickened. No interference with any of the functions.

March 13. Animal lively, apparently as well as ever.

Experiment 129:

March 15, 1875. Injected following solution by six different punctures over back and belly: Salicylic acid, 10 grains; sodic sulphate, 30 grains; water, 100 minims. The injecting occupied seven minutes. Immediate effects generally similar to those in Experiment 128, but shock-like condition more pronounced.

March 16. Animal lively and well.

Experiment 130:

March 16, 1875. Injected into same rabbit used in Experiment 129, the following solution by three different punctures: Salicylic acid, 10 grains; sodic phosphate, 30 grains; water, 50 minims. Animal became soon quiet and dull, but in half an hour brightened somewhat. One hour after the injections, fell upon side, unable to stand. Fifteen minutes later opisthotonic convulsions set in, and for the following hour the condition remained the same.

March 17, 8 o'clock A. M. Found dead and stiff. *Autopsy* at 3.15 o'clock P. M., just twenty-four hours after the injections: Connective-tissue under skin of thorax and abdomen infiltrated with serum, striking a violet color on addition of solution of ferric chloride (a test for salicylic acid). Ecchymoses at sites of injection. On opening abdominal cavity it was found that the needle of the injecting syringe had pierced the thin wall of the abdomen and wounded the peritoneum and liver. No other lesion, traumatic or otherwise, of any abdominal or thoracic organ, nor of the central nervous system. Bladder distended with four fluid drachms of clear urine, giving deep violet color on addition of the ferric salt. Contents of small intestine and alcoholic extract of the brain also give the reaction with iron, but not the contents of the stomach, nor the cut surface of the lungs, nor blood taken from the heart.

Experiment 131:

March 17, 1875. Same procedure as in Experiment 130, care being taken not to pierce the abdominal walls. Animal quiet and dull shortly after the injections, but not seriously affected.

March 18. Animal lively and well.

Experiment 132:

March 17, 1875. Injected following solution by seven different punctures. Salicylic acid, 15 grains; sodic phosphate, 45 grains; water, 75 minims. Immediate effects similar to those of Experiment 131.

March 18. Animal lively and well.

Experiment 133:

March 19, 1875. Injected following solution by ten separate punctures. Salicylic acid, 24 grains; sodic phosphate, 72 grains; water, 160 minims. The injecting occupied half an hour. Animal after last injection quiet and dull with increased rapidity of respiration. Fifteen minutes later, fell over on side with legs rigid and head thrown

back, and in seven minutes thereafter died. *Autopsy* immediately: Connective-tissue at most points of injection infiltrated with blood, with many ecchymoses in the neighborhood; coagula of a dirty reddish-brown on the edges. No lesions of any organ, traumatic or otherwise. Urine in bladder, fæces in small intestine, blood in heart, aqueous humor, alcoholic decoction of brain, all give a violet color on addition of solution of ferric chloride.

c. Hypodermic injection of simple aqueous solution of sodic phosphate, and of pure water as control experiments to the foregoing.

Experiment 134:

March 15, 1875. Injected the following solution by six separate punctures: sodic phosphate, 30 grains; water, 100 minims (same solution as in Experiment 129, only minus the salicylic acid). Results same as in Experiment 129.

Experiment 135:

March 16, 1875. Same procedure and results as in Experiment 134.

Experiment 136:

March 16, 1875. Injected by six separate punctures 100 minims of pure water. Animal quiet and dull for fifteen minutes.

March 17. Animal lively and well.

Experiment 137:

March 24, 1875. Injected the following solution by ten separate punctures: sodic phosphate, 72 grains; water 160 minims (same solution as in experiment 133 only minus the salicylic acid). Animal became immediately quiet and dull, breathing fast. Two hours later fell over on side in convulsions; legs rigid and twitching, but in seven minutes regained the feet and remained sitting quietly.

March 25 (twenty-four hours after last observation). Has eaten nothing; sits heavy and dull with eyes half closed; on pushing gently, fell over on side and pawed ineffectually to regain feet. Died quietly half an hour later.

March 26. *Autopsy:* No lesion of any organ, traumatic or otherwise, but subcutaneous connective-tissue deeply and generally infiltrated with serum.

VII.—INOCULATIONS OF SALICYLIC ACID AND VACCINE VIRUS.

Through the kindness of Dr. Frank P. Foster these inoculations were made at his establishment by Dr. W. F. Cushman and Mr. H. Lawrence, in presence of Dr. Satterthwaite. Solutions were prepared as follows: I. Salicylic acid, 1 part; distilled water, 500 parts. II. Salicylic acid, 1 part; sodic phosphate, 3 parts; distilled water, 250 parts. III. Sodic phosphate, 3 parts; distilled water, 250 parts. All being in readiness for the vaccinations, three portions of pure fresh vaccine lymph were mixed with equal volumes of these solutions, respectively, and the mixtures allowed to stand one minute before being used for vaccination.

Experiment 138:

March 28, 1876. A heifer was inoculated as follows: At four points with pure fresh vaccine lymph; at four points with lymph mixed with solution I. (making a dilute lymph impregnated with salicylic acid in the proportion of 1 to 1,000 parts); at four points with lymph mixed with solution II. (making lymph containing salicylic acid 1 part to 500); and at five points with lymph mixed with solution III.

April 4. Fine pocks produced in two out of the four inoculations of pure lymph; in all four inoculations of lymph with salicylic acid 1 to 1,000; in all four inoculations of lymph with salicylic acid 1 to 500; and in four out of the five inoculations of lymph with simple sodic sulphate solution. In all cases the pocks produced by the adulterated lymph were as fine as those produced by the pure, and were in all respects typical vaccine pocks.

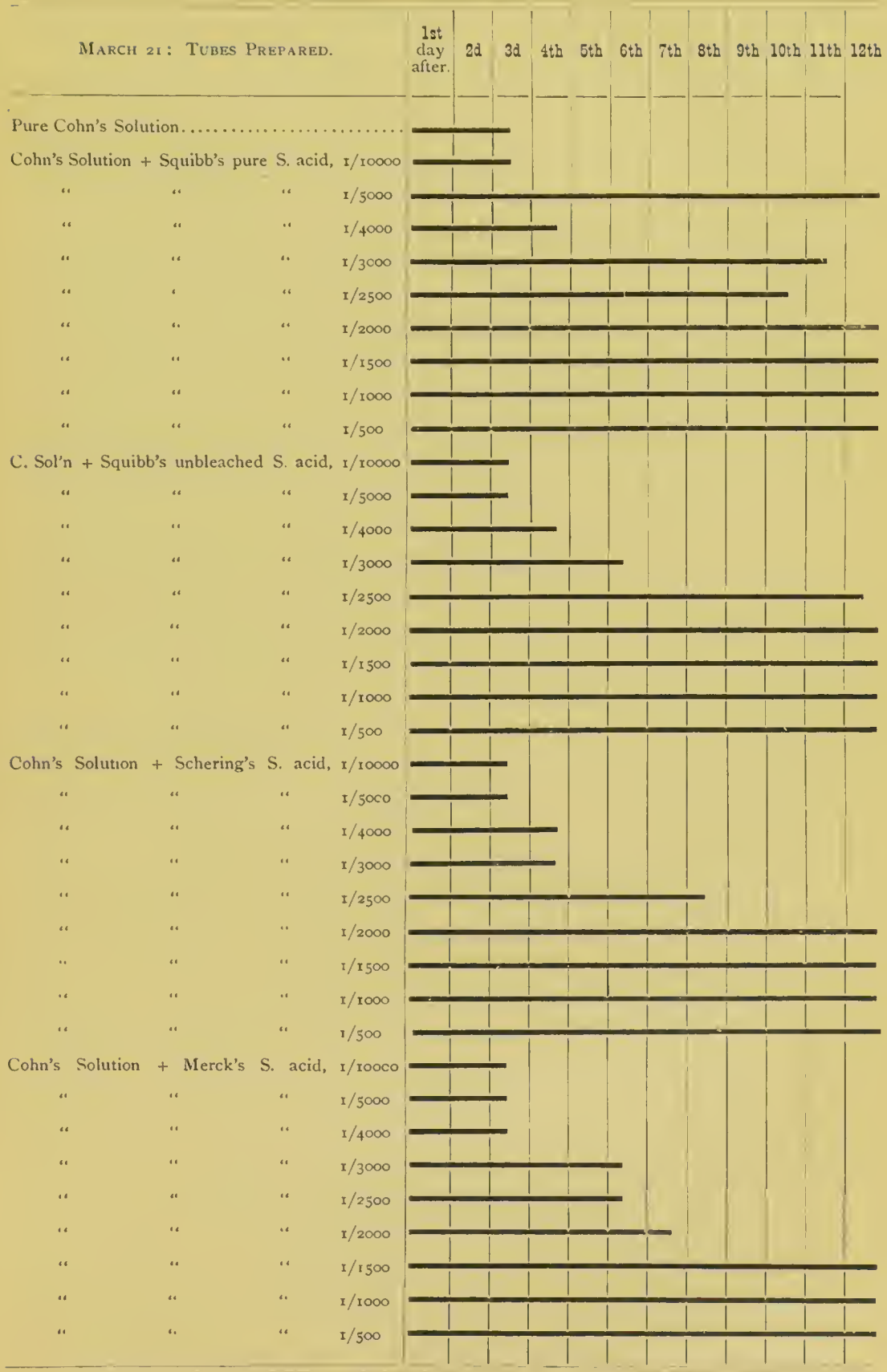
VIII.—EXPERIMENTS TO TEST THE POWER OF SALICYLIC ACID TO PREVENT THE DEVELOPMENT OF BACTERIA IN PUTREFIABLE FLUIDS.

In these experiments equal volumes of clear putrefiable fluids in test-tubes were charged with various percentages of the substances whose power was to be tested, and the tubes were then inspected from day to day, or as often as occasion permitted, and the first appearance of *cloudiness* of the contained fluid noted as a sign that bacterial development was beginning.

Experiments 139 to 175 inclusive:

March 21, 1875. Thirty-six test-tubes of Cohn's solution were charged with different proportions of salicylic acid of different makers, as in the following table. A test-tube of Cohn's solution, unadulterated, was placed among the rest. The whole were set upon a warm mantel-piece, and watched for twelve days. The lines indicate the number of days that the contents of the tubes remained clear.

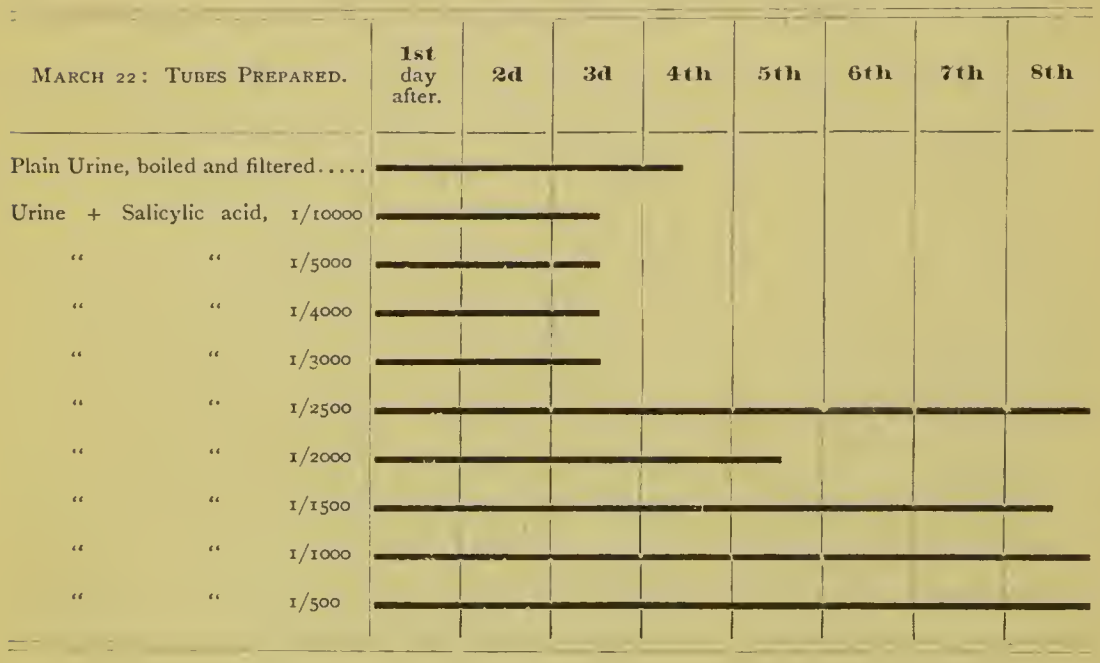
Cohn's Solution and Salicylic Acid.



Experiments 176 to 186 inclusive:

March 22, 1875. Fresh human urine was boiled, filtered, and then charged with Squibb's unbleached salicylic acid, as per table below. The test tubes were observed for eight days.

Urine and Salicylic Acid.



Experiments 187 to 196 inclusive:

April 29, 1875. The following aqueous solutions of Squibb's pure salicylic acid were made by the addition of a little sodic phosphate: 1: 100, 250, 500, 750, 1,000, 1,500, 2,500, 3,000, 5,000, 10,000. Two fluid drachms of each solution were put into a test-tube, and then the test-tubes were severally inoculated with one drop each of a strong, putrid beef-decoction. The tubes were finally loosely stopped with cotton. The tubes were examined at intervals, with the following result: All the solutions weaker than 1-1500 began to turn cloudy almost immediately. The following solutions were found cloudy on the days stated respectively: 1-1500 on the 6th day, 1-1000 on the 28th; 1-750 and 1-500 were clear on the 28th day, but were found cloudy on the 74th, no observation having been made between these dates; 1-250 and 1-100 remained clear during the whole 78 days of the observation.

Experiments 197 to 206 inclusive:

April 29, 1875. Precisely similar experiments were made with Squibb's unbleached salicylic acid. The results were practically identical with the foregoing.